

RESEARCH PAPER

No increase in alpine snowbed productivity in response to experimental lengthening of the growing season

F. Baptist¹, C. Flahaut¹, P. Streb² & P. Choler^{1,3}

1 Laboratoire d'Ecologie Alpine, UMR 5553 CNRS-UJF, Université de Grenoble, Grenoble, France

2 Laboratoire Ecologie, Systématique et Evolution, UFR Scientifique d'Orsay Université Paris Orsay, Orsay, France

3 Station Alpine J. Fourier, UMS 2925 CNRS-UJF, Université de Grenoble, Grenoble, France

Keywords

Alopecurus gerardi; alpine tundra; *Carex foetida*; early frost; growing season length; *Poa alpina*.

Correspondence

F. Baptist, Laboratoire d'Ecologie Alpine, UMR 5553 CNRS-UJF, Université de Grenoble, BP 53, F-38041 Grenoble Cedex 09, France.
E-mail: florence.baptist@ujf-grenoble.fr

Editor

J. Sparks

Received: 27 February 2009; Accepted:
5 October 2009

doi:10.1111/j.1438-8677.2009.00286.x

ABSTRACT

Climate change effects on snow cover and thermic regime in alpine tundra might lead to a longer growing season, but could also increase risks to plants from spring frost events. Alpine snowbeds, *i.e.* alpine tundra from late snowmelt sites, might be particularly susceptible to such climatic changes. Snowbed communities were grown in large monoliths for two consecutive years, under different manipulated snow cover treatments, to test for effects of early (E) and late (L) snowmelt on dominant species growth, plant functional traits, leaf area index (LAI) and aboveground productivity. Spring snow cover was reduced to assess the sensitivity of snowbed alpine species to severe early frost events, and dominant species freezing temperatures were measured. Aboveground biomass, productivity, LAI and dominant species growth did not increase significantly in E compared to L treatments, indicating inability to respond to an extended growing season. Edapho-climatic conditions could not account for these results, suggesting that developmental constraints are important in controlling snowbed plant growth. Impaired productivity was only detected when harsher and more frequent frost events were experimentally induced by early snowmelt. These conditions exposed plants to spring frosts, reaching temperatures consistent with the estimated freezing points of the dominant species (~ -10 °C). We conclude that weak plasticity in phenological response and potential detrimental effects of early frosts explain why alpine tundra from snowbeds is not expected to benefit from increased growing season length.

INTRODUCTION

The latest climate change predictions from the IPCC for 2080 estimate that mountainous regions from the northern hemisphere will be subject to an increase in mean annual temperature and to changes in patterns of precipitation, soil moisture and snow cover. The predicted decrease in snowfall (Serreze *et al.* 2000; Dye & Tucker 2003; Beniston 2005), combined with increased temperature (Beniston 2005; Noguès-Bravo *et al.* 2007), is likely to bring forward spring snowmelt, and consequently affect soil temperature and moisture regimes. Reduced snow cover during winter associated with earlier snowmelt would result in longer growing seasons and higher cumulative thawing degree-days (TDD), which could

stimulate vegetation growth (Theurillat & Guisan 2001). However, the absence of insulating snow cover and earlier dehardening could expose plants to higher risk of frost damage, and an extended growing season could lead to late-summer water stress (Inouye 2000; Wipf *et al.* 2006; Björk & Molau 2007; Edwards *et al.* 2007). Alpine snowbed species, *i.e.* alpine species from late snowmelt sites, might be particularly vulnerable to such changes (Schöb *et al.* 2009).

In snow-covered ecosystems, the growing season is generally constricted at both ends by subzero temperatures. The time available for growth largely determines aboveground biomass and primary productivity, which feed back on nutrient availability. It also affects community composition by differentially altering the performance of

species with different growth and reproductive strategies (Molau 1993; Galen & Stanton 1993). A large number of studies have experimentally addressed the effects of warming (Jones *et al.* 1997; Molau 1997; Stenström & Jónsdóttir 1997; Stenström *et al.* 1997; Welker *et al.* 1997; Price & Waser 2000; Starr *et al.* 2000; de Valpine & Harte 2001; Dunne *et al.* 2003; Saavedra *et al.* 2003) and early or delayed snowmelt (Scott & Rouse 1995; Dorrepaal *et al.* 2003; Dunne *et al.* 2003; Saavedra *et al.* 2003; Wahren *et al.* 2005) on plant growth and phenology of particular arctic and alpine species. Some of these studies have found that plants flower earlier in response to a longer growing season and higher cumulative TDD (but see Molau *et al.* 2005). However, these studies only considered snowmelt gradients *in natura* or performed experiments with artificial warming, leading to an increase in air temperature at snowmelt. Furthermore, only a few studies have addressed the effects of shallower snowpack and reduced snowcover duration on aboveground biomass or productivity of snowbed tundra in temperate alpine ecosystems (Starr *et al.* 2000; Wipf *et al.* 2006, 2009). How changes in growing season length impact plant productivity remains largely unknown, although this knowledge is crucial for predicting the consequences of global climate change on species distribution and carbon balance of snow-covered ecosystems.

The first aim of this study was to examine the effects of a reduced snowcover period on the phenology and growth of snowbed alpine species by experimentally manipulating winter snow cover duration. We compared the effects of an early (E) and a late (L) snowmelt on aboveground biomass, integrated productivity and leaf area index (LAI) of snowbed communities. Changes in plant functional trait values and growth phenology of the dominant species were also investigated. The plant functional traits measured were specific leaf area (SLA), leaf dry matter content (LDMC) and leaf nitrogen content (LNC), as these are related to carbon fixation and productivity (Garnier *et al.* 2004; Quétier *et al.* 2007). Because a shortened snow cover period means a higher risk of early frosts, we further assessed sensitivity of the snowbed community to harsher and more frequent frost events. For this purpose, we experimentally forced the system by strongly limiting snow cover very early in spring, and measured freezing temperature and frost tolerance of the three dominant species of the snowbed community.

MATERIALS AND METHODS

Study site and species

The investigated snowbed communities were dominated by graminoids: *Carex foetida* (Cyperaceae), *Alopecurus gerardi* (Poaceae) and *Poa alpina* (Poaceae). *C. foetida* and *A. gerardi* can be considered snowbed specialists in temperate alpine tundra, whereas *P. alpina* is a generalist species. These plant communities correspond to one of

the four main types of snowbed community in the southwest Alps and Apennines (Tomaselli 1991). Soils are generally deep (>1 m) and enriched with clay; organic matter content of the soil is $8 \pm 0.3\%$ in the top 20 cm. Despite the shortened growing season, aboveground primary productivity is high (Choler 2005; Baptist & Choler 2008; see also Fig. S1 in the Supporting information section).

On 27 September 2005, 15 monoliths (25-cm deep and 45×35 cm surface) were randomly selected and then excavated from a large snowbed situated near the Agnel Pass in the southwest French Alps (2744 m asl). The mean snowmelt time of this site is early June (Choler, personal observations). Monoliths were transferred into plastic pots with drainage and transported to the (outdoor) experimental garden of the Station Alpine J. Fourier, an alpine field research station at the Lautaret Pass (2100 m asl, $45^{\circ}7'N$, $6^{\circ}5'E$). Because we excavated whole monoliths without disrupting root systems, we believe that any disturbance effect was minimal. Relative abundance of *C. foetida* accounted for more than 50%, *A. gerardi* 31%, other graminoids 3% and forbs 14% (see Table 1).

Experimental design and treatments

At the end of September 2005, at the alpine field station, the five monoliths were randomly allocated to each of the following three treatments: (i) a limited winter snowpack, where monoliths were disposed on the summit of a hill-ock (inconsistent snow cover treatment, I); (ii) a winter snow-covered but early snowmelt treatment (early snow cover treatment, E); and (iii) a delayed snowmelt treatment, where 1–2 m of snow were added to monoliths, and these were placed in the shade to delay snowmelt (late snow cover treatment, L). All sets of monoliths were 5–10 m away from each other and were buried in the soil. First snowfalls occurred on the 1 December 2005. In order to avoid microsite effects during the growing season (from snowmelt to the end of October), all the monoliths were transferred to a common location in the common garden of the alpine research station and again buried in

Table 1. Relative abundance (%) of *Carex foetida*, *Alopecurus gerardi* and two functional groups reported as 'Other graminoids' and 'Forbs' in 2006 and 2007 in response to snow treatments. In 2007, the group 'Other graminoids' was mainly represented by *P. alpina*. Values are mean \pm standard error (SE). See text for statistical details.

species/groups		late	early	inconsistent
<i>C. foetida</i>	2006	53 (5)	57 (4)	55 (3)
	2007	39 (7)	50 (4)	46 (5)
<i>A. gerardi</i>	2006	31 (7)	29 (4)	27 (4)
	2007	33 (7)	28 (2)	19 (5)
Other graminoids	2006	3 (1)	7 (2)	4 (3)
	2007	14 (1)	20 (5)	24 (5)
Forbs	2006	14 (5)	6 (5)	11 (4)
	2007	14 (4)	3 (2)	10 (5)

the soil. The same snow cover treatments were repeated during the winter 2006–2007.

Treatment L resulted in a snowmelt time as close as possible to that of the native site. After 1 and 2 years of the experiment, the physiognomy and floristic composition of all monoliths remained very comparable to native snowbed communities. In addition, integrated primary productivity in all monoliths was very similar to measurements made in the field. These factors strongly minimise the risk that observed responses are just experimental artifacts.

Edapho-climatic recordings

Hourly soil temperature was recorded during the 2005–2006 and 2006–2007 winters for each snow treatment using Hobo probes (Onset Computer Corp., Bourne, MA, USA) buried 5 cm below the ground. Consistent day temperatures close to 0 °C (usually between –1 and 1 °C) were considered indicative of persistent snow cover. In the E and L treatments, the start of the growing season was considered as the last day of persistent snow cover, which correlates with a marked amplitude in daily course of soil temperature and a daily maximum temperature above 0 °C. In the I treatment, the start of the growing season was only referred to the daily thermal amplitude of the soil and maximal diurnal soil temperature above 0 °C. Cumulative thawing degree-day (TDD) was calculated as the sum of daily mean temperatures above 0 °C from the start of the growing season. To compare snowmelt dates and climatic conditions in the field and in the common garden, we recorded soil temperature at four snowbeds in the Combe Roche Noire valley (2550 m asl) and close to the Galibier Pass (2600 m asl) from 2004 to 2007. In spring and early summer, the number of days when minimum air temperature was below 0 °C or –5 °C was calculated from hourly air temperature recordings at the Station Alpine J. Fourier from 2004 to 2007. An adiabatic gradient of 0.5 °C per 100-m elevation was used to take into account differences in elevation between the recording site and the alpine snowbed location (mean elevation 2600 m asl). Gravimetric soil water content was measured in each monolith at peak standing biomass in July 2006 and 2007. Simultaneously, we measured volumetric soil moisture using time domain reflectometry (TDR probes, mini TRASE system 1, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) every 1 to 4 weeks in each monolith at the beginning of the growing season in 2007 (see Fig S2 in the Supporting information section). Finally, we used PAR light sensors located in the common garden to estimate cumulative mean global radiation.

At peak standing biomass during the 2006 and 2007 growing seasons, three soil cores (1-cm diameter) were extracted from each monolith and pooled for analysis of total inorganic nitrogen content. Nitrogen was extracted from fresh soil samples (~10 g) with 2 M KCl after sieving at 2 mm. Soil extracts were analysed for ammonium

(NH₄⁺) and nitrate/nitrite (NO₃⁻/NO₂⁻) content using an FS-IV autoanalyser (OI-Analytical, College Station, TX, USA).

Plant responses

Species abundance, biomass and plant functional traits were recorded at peak standing biomass on 15 July 2006 and on 3 July 2007. The mean cover of plants was estimated using two line transects per monolith, with a plant contact recorded every 2 cm for a total of 50 points per monolith. The mean cover of a species corresponded to the area of ground covered by the vertical projection of its green aerial parts. In each monolith, total standing biomass was collected in a randomly located square plot of 5 × 5 cm. Integrated productivity was determined by dividing peak standing biomass (dried at 60 °C for 48 h) by cumulative TDD between snowmelt and peak biomass. This estimate corresponds to seasonally averaged above-ground productivity. LAI was determined by measuring total projected area of green leaves with a leaf area meter (WinDIAS, Delta-T Device Ltd., Cambridge, UK).

Species selected for trait measurement were *C. foetida*, *A. gerardi* and *P. alpina* (the last species only measured in 2007). They accounted for more than 70% of the maximum standing live biomass of the community. Specific leaf area (SLA), leaf dry matter content (LDMC) and leaf nitrogen content (LNC) were measured following standard protocols (Cornelissen *et al.* 2003) on cohort 2 in the case of *C. foetida* (see below) and on mature, fully expanded leaves in the case of *A. gerardi* and *P. alpina*. Briefly, three sets of leaves were collected in each monolith and maintained in moist paper. After rehydration for 6 h, they were weighed and total surface measured using a leaf area meter (Gatehouse Scientific Instruments Ltd., Norfolk, UK), dried for 48 h at 60 °C and weighed for dry mass. Leaves were ground and analysed for carbon and nitrogen using a CHN analyser (Thermo Electron Corp., Madison, USA).

Diachronic study of *C. foetida* growth

We monitored leaf elongation of the dominant sedge *C. foetida*, during the 2006 and 2007 growing seasons, from snowmelt until senescence, on three randomly marked tillers per monolith. We considered this measurement as an adequate surrogate of leaf growth, as leaf width was strongly correlated to leaf length (F. Baptist, personal observation). Tillers are monocarpic, and leaves senesce rapidly if an inflorescence is produced. However, it was impossible to distinguish vegetative and flowering tillers at snowmelt, and reproductive tillers were excluded *a posteriori*. We measured green and total leaf length from the oldest to the youngest leaf every 2–4 weeks. The difference due to senescing tips increased over the growing season (see Shaver & Laundre 1997 for a precise description of the internal dynamic of leaf growth in the genus *Carex*). The species produced on average nine–11 leaves

per year from the basal meristems. We pooled leaves into three cohorts (three leaves per cohort). Cohort 1 corresponded to leaves that were initiated in the previous summer and displayed weak growth at the beginning of the growing season (senescent fraction >50%); leaves in cohort 2 were initiated at snowmelt; and leaves in cohort 3 were initiated later in the growing season. To adequately compare treatments, we expressed growth as a function of cumulative TDD and as a function of the number of elapsed days after snowmelt.

Freezing point and frost tolerance

In June 2007, just after snowmelt, three vegetation cores were collected in snowbeds near the Galibier Pass (2650 m asl). During the following 2 days, freezing temperatures of *C. foetida*, *A. gerardi* and *P. alpina* leaves were measured ($n = 3$ for *A. gerardi* and 4 each for *C. foetida* and *P. alpina*). Two thermocouples (0.5-mm and 1.0-mm diameter for leaf and air temperature, respectively) were connected to a CR800X Campbell data logger (Campbell Scientific Inc., Logan, UT, USA), recording every 5 s. Maximum, minimum and mean temperatures were recorded every 2 min. Freshly cut leaves were placed in contact with the leaf thermocouple in a closed metal chamber cooled with antifreeze. The temperature inside the chamber was decreased progressively, using a programmed water bath, at a rate of 1 °C every 5 min to a final leaf temperature of -15 °C and then increased up to 25 °C. Late treatments were carried out with temperatures declining at 2 °C·h⁻¹. At their freezing point, leaf temperatures increased due to heat emission, which was detected by the first thermocouple (Ball *et al.* 2002). Parallel measurements of fluorescence also indicated freezing temperatures, through a strong increase in minimal fluorescence F_0 (Neuner & Pramsöhler 2006). To confirm freezing-induced damage, the integrity of leaf cells was assayed, directly before and after determining the freezing temperature, as the percentage change in conductivity following the incubation of leaves in deionised water at room temperature for 24 h, before and after breakage of cells by boiling (10 min) (Webb *et al.* 1996).

Statistical analysis

Repeated-measures analysis of variance (RMANOVA) was carried out to compare overall differences caused by the snow treatments (between-subject effects) and the interaction between treatments and time (within-subject effects) on aboveground biomass, integrated productivity, LAI and leaf traits. A one-way ANOVA was used to test for the effects of snow treatments on *P. alpina* leaf functional traits (measured only in 2007). In all cases, individual monoliths were treated as replicates, and tiller-based variables were analysed after averaging per monolith. Similarly, differences in the seasonal growth of *C. foetida* leaves between snow treatments were compared only at maximal growth using a RMANOVA. The variable analysed

was mean green leaf length (*i.e.* length of only the green part of the leaf) of each cohort averaged over the three tillers selected in each monolith. Freezing temperatures and integrity of cells were analysed using the non-parametric Kruskal–Wallis test. All statistical analyses were performed with the Jump software (SAS Institute Inc, Cary, NC, USA).

RESULTS

In the common garden, the L (late snow cover treatment) monoliths experienced a slightly shorter winter than in the field. In the corresponding alpine snowbeds (between 2500 and 2700 m asl), snowmelt occurred in mid-June (see Table S1 for snowmelt dates in the field from 2004 to 2007) compared to 8 June 2006 and 15 May 2007 in the common garden (Table 2). Cumulative TDD reached on average 1200 in the field compared to more than 2000 in the common garden. However, at peak standing biomass, cumulative TDD between the L treatment and the field did not differ, and was approximately 600 cumulative TDD (see Baptist & Choler 2008). The number of days when the minimal daily air temperature was below 0 °C was still between 3 and 8 days during the first 10 days of June (Fig. 1). Daily minima below -5 °C were also frequent at the end of May and at the beginning of June, corresponding to snowmelting dates of those years with a particularly warm spring, or reduced winter snowpack (*e.g.* in 2006 and 2007, Fig. 1 and Table S1).

Effects of snow treatments on edaphic conditions

During the growing season (defined as the period where soil temperature was >0 °C), the E (early snow cover treatment), I (inconsistent snow cover treatment) and L monoliths experienced similar temperature and water regimes ($F_{2,12} = 1.0$, $P = 0.39$) (Table 2, Fig. 2 and Fig. S3). Seasonal records of soil moisture during 2007 did not reveal any differences between snow treatments (see Fig. S2). There was no effect of snow treatments on $[\text{NH}_4^+]$ at peak standing biomass ($F_{2,10} = 1.2$, $P = 0.34$), but we did detect a significant year effect ($F_{1,10} = 13.2$, $P = 0.001$), indicating higher $[\text{NH}_4^+]$ concentration in 2007 compared to 2006. In contrast, $[\text{NO}_3^-]$ was significantly affected by the snow treatments ($F_{2,10} = 6.3$, $P = 0.02$), as was the interaction ($F_{2,10} = 6.0$, $P = 0.02$), indicating inconsistent patterns in response to snow treatments over the 2 years: $[\text{NO}_3^-]$ was highest in L monoliths in 2007 and lowest in 2006. Finally, a significant year effect was detected, with higher $[\text{NO}_3^-]$ concentrations in 2007 than in 2006 ($F_{1,10} = 21.6$, $P < 0.001$).

Plant responses to early and late snow cover treatments

Snowmelt in the E treatment advanced by 24 and 21 days in 2006 and 2007, respectively, compared to the L treatment. Cumulative TDD (2006/2007) at biomass peak in the E treatment experienced an increase of 208 and 149

Table 2. Edapho-climatic conditions experienced by monoliths in response to snow treatments in 2006 and 2007: (a) time, (b) thermic regime, (c) solar radiation, (d) water status measured at mid-season, and (e) soil fertility. The number of frost events was counted, starting from the date at which air temperature was considered sufficiently high to trigger growth in the different treatments. Mean \pm standard deviation (SD) in the case of mean winter soil temperature, mean \pm standard error (SE) for water status and soil fertility. See text for statistical details.

	year	late	early	inconsistent
(a) time				
snowmelt (date)	2006	8-June	15-May	14-April
	2007	15-May	24-April	3-April
(b) thermic regime				
cumulative TDD at: Biomass peak/End of season	2006	552/1685	760/1890	974/2067
	2007	597/1806	746/1955	893/2102
mean wintertime soil temperature ($^{\circ}$ C)	2006	-0.8 (1)	-0.9 (1.0)	-3.3 (3.4)
	2007	0.3 (1.5)	0.3 (1.6)	-0.4 (2.3)
number of frost events (≤ 0 $^{\circ}$ C)/Minimum air temperature ($^{\circ}$ C)	2006	0/-	6/-5.7	28/-8.1
	2007	6/-4.4	9/-4.4	21/-13.1
(c) solar radiation				
cumulative daily mean solar radiation from snow melt to	2006	1431	2433	3641
peak standing biomass (mol photons $m^{-2}\cdot day^{-1}$)	2007	1908	2598	3385
(d) water status				
soil moisture measured at mid-season (mass %)	2006	22.0 (1.5)	25.0 (2.4)	29.0 (1.7)
	2007	26.1 (3.5)	27.6 (3.8)	29.4 (2.3)
(e) soil fertility (mid-season)				
NO ₃ (mg-g ⁻¹ soil DW)	2006	0.32 (0.25)	0.63 (0.31)	0.45 (0.12)
	2007	3.1 (0.4)	0.92 (0.11)	1.7 (0.4)
NH ₄ (mg-g ⁻¹ soil DW)	2006	10.2 (1.1)	9.5 (2.1)	11.2 (2.3)
	2007	8.7 (0.8)	5.3 (0.4)	5.1 (0.9)

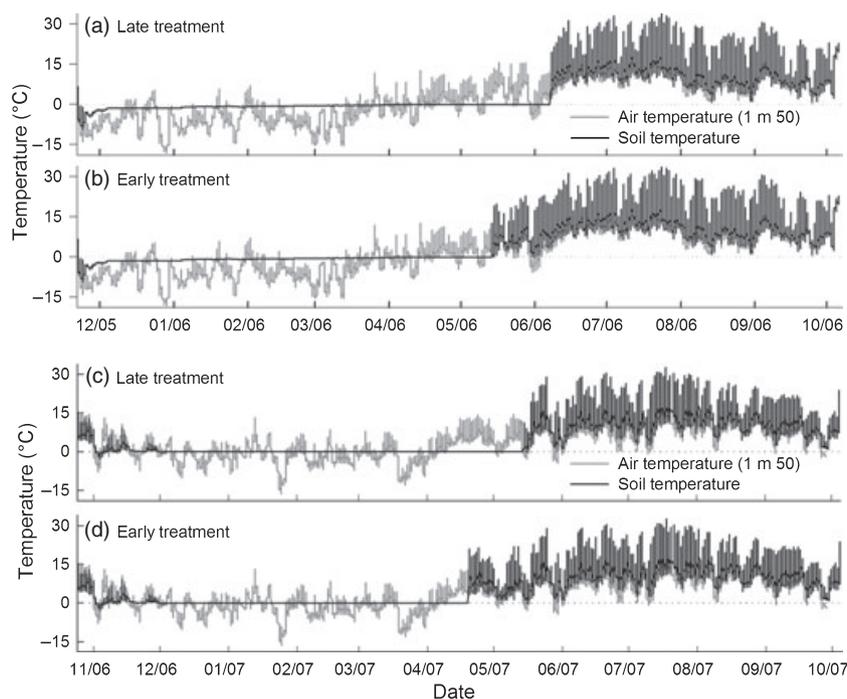


Fig. 1. Daily mean soil (5-cm deep) and air temperature (grey) in the L and E treatments in 2005–2006 (a,b) and 2006–2007 (c,d).

degree-days in 2006 and 2007, respectively, compared to the L treatment (Table 2). The relative abundance of *C. foetida* and *A. gerardi* did not respond to the E and L treatments (respectively $F_{1,8} = 1.14$, $P = 0.31$; $F_{1,8} = 0.15$,

$P = 0.70$; Table 1). Similarly, the relative abundance of others graminoids (mainly *P. alpina*) and forbs did not exhibit consistent patterns ($F_{1,8} = 1.72$, $P = 0.22$; $F_{1,8} = 3.44$, $P = 0.10$). *Poa alpina* showed a year effect,

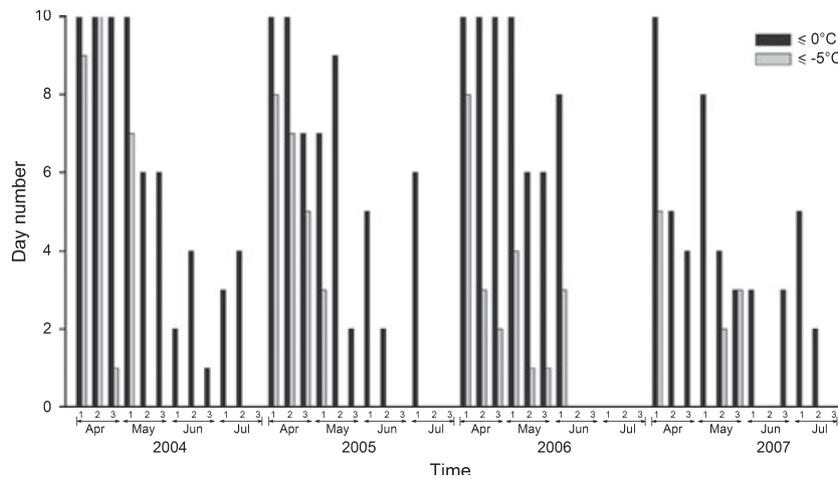


Fig. 2. Number of days (per 10, three sets per month) when daily minimal air temperature was below 0 °C (black bar) or -5 °C (grey bar) at Galibier Pass (at 2600 m asl, France), from April until July for the years 2004 to 2007. Air temperatures were measured at the Lautaret Pass (2100 m asl) 5 km from the study site. A temperature adiabatic gradient of 0.5 °C per 100 m elevation was used to account for the differences in elevation. See Material and Methods section for more details.

with an increase in 2007 ($F_{1,8} = 81.44$, $P < 0.0001$) irrespective of treatment. The treatments \times year interaction was not significant ($P > 0.48$ for all species and plant groups).

Aboveground biomass between the E and L monoliths was not significantly different ($P = 0.31$; Fig. 3a). This indicates that the significant difference in integrated productivity was a result of differences in the duration of snow cover rather than the rate of biomass production ($F_{1,8} = 17.18$, $P < 0.01$; Fig. 3b). A year effect was detected ($F_{1,8} = 6.43$, $P = 0.03$, $F_{1,8} = 7.66$, $P = 0.02$), indicating higher biomass and integrated productivity in 2007 compared to 2006. The interactions were not significant ($P > 0.60$). Similar to aboveground biomass, LAI was lower in the E compared to the L treatment but this effect was not statistically significant ($P = 0.09$; Fig. 3c), and overall LAI was significantly higher in 2007 ($F_{1,8} = 7.69$, $P = 0.02$) than in 2006. The treatment \times year interaction was not significant ($P = 0.39$).

The accumulated leaf length of cohort 2 accounted for more than $46 \pm 1\%$ of total accumulated leaf length of the tillers, whereas cohorts 1 and 3 accounted for $22 \pm 1\%$ and $32 \pm 1\%$, respectively. Maximal green leaf length for cohort 1 did not differ between the E and L treatments ($P = 0.66$), indicating that growth was not enhanced by a longer growing season (Fig. 4). Leaves required similar cumulative TDD to reach maximal biomass and, hence, the date of peak standing biomass was slightly later in the L monoliths. Snow treatments resulted in significant differences in maximal green leaf length of cohort 2 ($F_{1,8} = 18.40$, $P < 0.01$; Fig. 4): *C. foetida* leaves in E monoliths had lower maximal green leaf length than in the L monoliths. At peak biomass, leaves from cohort 3 (initiated later in season) did not show significant differences in their patterns of response to snow treatment ($P = 0.22$). SLA, LDMC and LNC of *C. foetida*, *A. gerardi* and *P. alpina* did not differ between the E and L treatments (Table 3). We detected a significant year effect in the case of SLA for *C. foetida* and *A. gerardi*, which was nevertheless not consistent: SLA of *C. foetida* was higher

in 2007 than in 2006 ($F_{1,8} = 14.21$, $P < 0.01$), whereas the opposite was true for *A. gerardi* ($F_{1,8} = 22.44$, $P < 0.01$).

Plant responses to inconsistent snow cover treatment

In order to assess the sensitivity of snowbed communities to frost events, we limited snow accumulation during winter (I treatment), which resulted in an increased number of frost events very early in the season (see Table 2 and Fig. S3 for climatic data). Typically, at the end of the winter period 2005–2006, I monoliths experienced 28 freezing events, with minimal air temperatures as low as -8.1 °C (Table 2 and Fig. S3), whereas air temperature did not decrease below 0 °C in the L treatment (Table 2).

The relative abundance of *C. foetida* and *A. gerardi*, and of others graminoids and forbs did not change between the I and the L monoliths ($P > 0.19$; Table 1). Aboveground biomass and integrated productivity were significantly affected in the I treatment (respectively $F_{1,8} = 6.42$, $P = 0.03$ and $F_{1,8} = 44.9$, $P < 0.001$) and displayed a marked decrease in comparison to the L treatment (Fig. 3a, b). Similarly, LAI was negatively affected in the I treatment ($F_{1,8} = 15.62$, $P < 0.01$) over the two seasons (Fig. 3c). However, SLA, LDMC and LNC of *C. foetida*, *A. gerardi* and *P. alpina* did not differ from those of the L treatment (Table 3).

Leaf freezing temperatures of *C. foetida*, *A. gerardi* and *P. alpina* were -10.0 °C, -12.0 °C and -9.3 °C, respectively, and did not differ significantly (Kruskal–Wallis $\chi^2(2) = 5.59$, $P = 0.06$; Fig. 5). The integrity of cells, as indicated by electrolyte leakage, varied greatly between the three species, with less than 40% of cells destroyed in *P. alpina* tissue compared to more than 70% in *C. foetida* and *A. gerardi* (Kruskal–Wallis $\chi^2(2) = 7.84$, $P = 0.02$).

DISCUSSION

Despite an extended growing season, aboveground biomass and LAI in the E treatment were similar to those of

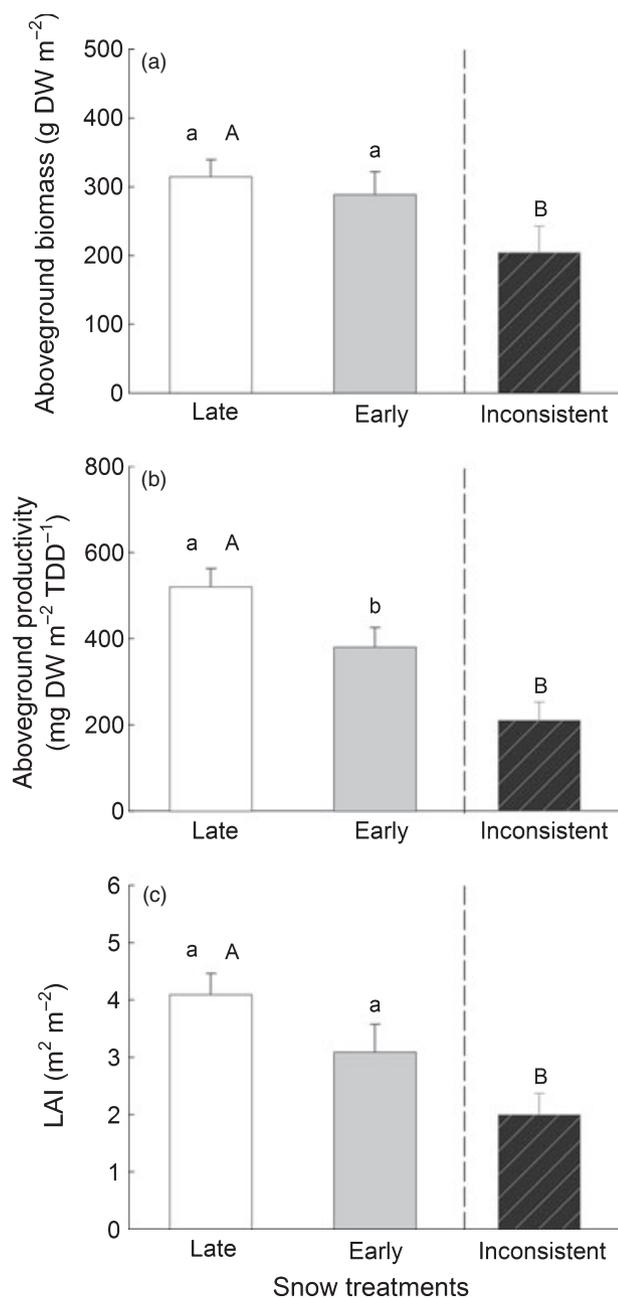


Fig. 3. Aboveground biomass (a), integrated aboveground primary productivity expressed per cumulative thawing degree-day (TDD) (b), and LAI (c) in response to snow treatments. Mean \pm SE for each treatment for both year ($n = 15$). Different letters refer to significant differences between L and E treatments (lowercase letters) and between L and I treatments (uppercase letters) ($P < 0.05$). See main text for more details on the statistical analysis.

the L treatment. As a result, integrated productivity (calculated from snowmelt date until peak standing biomass) was much lower in the E treatment, with a longer growing season compared to the L treatment. Similarly, the

diachronic records of *C. foetida* growth clearly demonstrated that annual production of leaf tissue was not enhanced by extending the growing season. Galen & Stanton (1993, 1995) postulated that because of lower temperatures, earlier snowmelt might limit respiratory costs and so further enhance growth. This clearly contrasts with the results obtained in here. Neutral and negative effects on plant growth of early snowmelt have been reported by Starr *et al.* (2000) and Wipf *et al.* (2006, 2009). Other studies have suggested a positive effect on plant cover (Galen & Stanton 1993, 1995; de Valpine & Harte 2001), but only considered snowmelt gradients *in natura* or performed experiments with artificial warming, leading to an increase in air temperature at snowmelt.

Various explanations can be proposed to explain lack of response in the E compared to the L treatment. E monoliths could be limited by water availability leading to reduced plant growth; however, soil moisture measured at peak standing biomass did not differ between the two treatments. Similarly, measurements throughout the season did not indicate differences in soil moisture between treatments in 2007 (Fig. S2). Finally, SLA and LDMC, which are good indicators of water stress (Cunningham *et al.* 1999; Wright *et al.* 2001; Gianoli 2004), did not differ between treatments, suggesting that soil moisture did not affect biomass production. Differences in fertility could also account for the results. Indeed, during winter, a deeper and longer-lasting insulating snowpack leads to higher microbial biomass and litter decomposition (Hobbie & Chapin 1996; Lipson *et al.* 1999). Furthermore, growth is often thought to start at snowmelt, through the percolation of melting water into the soil (Bowman 1992), and the death of microbial populations leads to an increase in N availability (Brooks *et al.* 1998). Thus, a shortened growing season might allow higher integrated aboveground productivity in the L treatment through increased soil fertility. However, despite a noticeable year effect, soil N concentrations measured at peak standing biomass could not consistently explain variations in aboveground, integrated productivity. Similar results were obtained at snowmelt in 2006 (data not shown). Total soil mineral N content was only from point measurements and might not be representative of the N available to plants during the growing season. However, the values of LNC tend to support the point data, as they were not affected by the snow treatments. This corroborates the findings of Jaeger & Monson (1992) and Starr *et al.* (2000), who showed no variation in LNC in response to warming or lengthened growing season. Spring growth of alpine plants is mainly sustained by the remobilisation of N out of storage structures (Lipson *et al.* 1996; Monson *et al.* 2006). These internal constraints buffer the interannual variability of N availability in the soil and might enable plants to sustain higher tissue production at similar N concentrations (Monson *et al.* 2006). Thus, neither fertility and water availability nor intraspecific changes in leaf trait values can consistently explain the lack of response of the snowbed com-

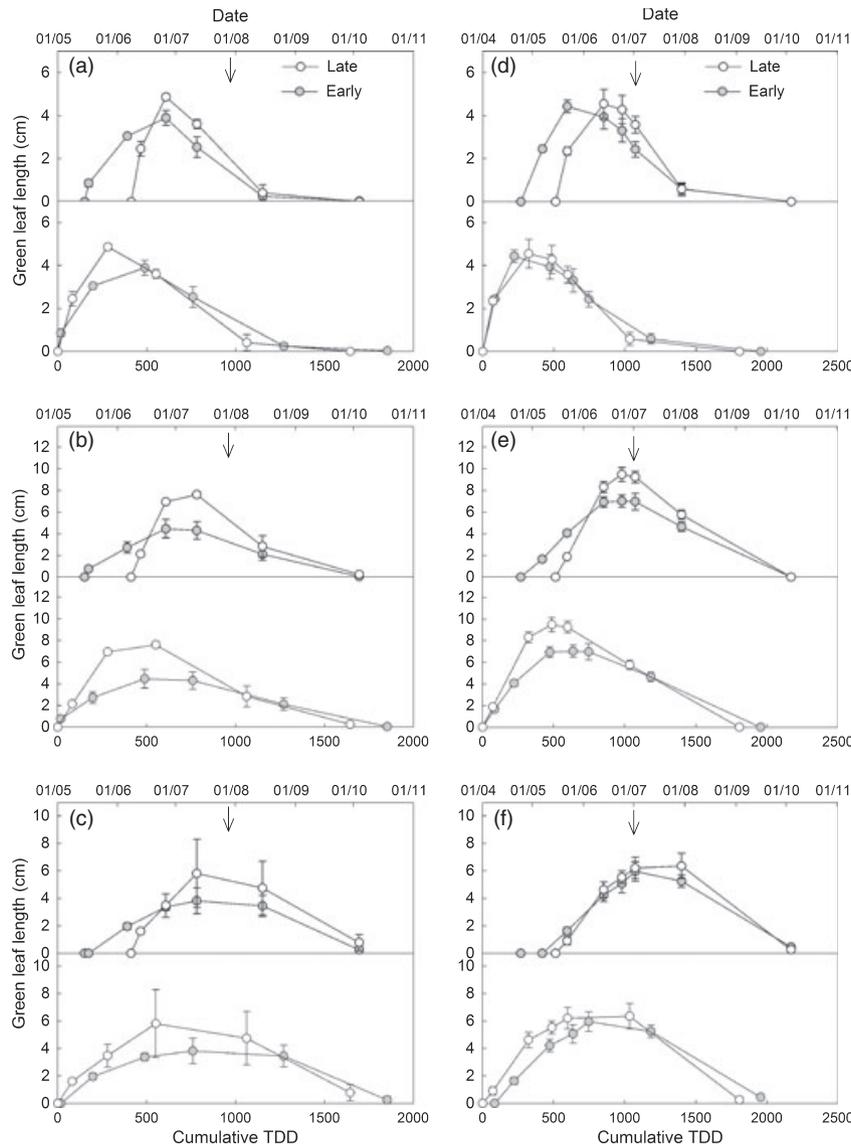


Fig. 4. Seasonal mean green leaf growth (cm) for three cohorts of *Carex foetida* leaves (cohorts 1, 2 and 3, see text for further details) related to Julian days (upper) and to cumulative thawing degree-days (lower, cumulative TDD) in 2006 (a, b, c) and 2007 (d, e, f) in the L and E treatments. Arrows indicate the day when aboveground biomass, LAI and leaf functional trait values were measured. See main text for more details on statistical analysis.

munity to an extended growing season. Hence, it appears that only developmental constraints can account for these results. Sørensen's classification (Sørensen 1941), further developed by Molau *et al.* (2005), distinguished two phenological patterns in tundra plants: periodic species, characterised by a fixed growing period, controlled by genetic constraints; and aperiodic species, for which the growth and production of new leaves proceeds when conditions become favourable. Accordingly, the snowbed species studied here, and in particular *C. foetida*, are periodic species and do not benefit from a lengthened growing season. Hence, these results indicate that the potential for the plants to increase productivity will strongly depend on their intrinsic developmental capacities (*e.g.* growth plasticity). Similar results were found by Wipf *et al.* (2009), who showed that plant responses to modifications in snow cover differ strongly among subalpine species.

The inconsistent snow cover in early spring (I treatment), associated with a higher frequency of frost events, resulted in a significant reduction in aboveground biomass, integrated productivity and LAI in the I treatment compared to the L treatment. Edapho-climatic conditions, such as water availability or soil fertility, and plant functional traits did not differ between I and L treatments. Developmental constraints might limit enhanced growth in response to an extended growing season, but cannot be responsible for lower aboveground biomass. Hence, frost events appear to be the only pertinent explanation for reduced integrated productivity in the I monoliths. The potential of frosts to damage alpine plants during the growing season has been reported (Körner 1999; Taschler & Neuner 2004). In early spring 2006 and 2007, the higher occurrence of severe frosts (\approx air temperature < -5 °C) in the I treatment (Table 2) associated with

Table 3. Specific leaf area (a), leaf nitrogen content (b) and leaf dry matter content (c) of the leaves of the three dominant species of snowbed sites (*Carex foetida*, *Alopecurus gerardi* and *Poa alpina*) in response to snow treatments. Leaf functional trait values for *Poa alpina* were only measured in 2007. Values are mean \pm standard error (SE). See text for statistical details.

	late	early	inconsistent
(a) specific leaf area (cm ² ·g ⁻¹)			
<i>C. foetida</i>	195.0 (7.2)	192.0 (3.3)	184.1 (3.6)
<i>A. gerardi</i>	181.4 (8.4)	173.0 (6.6)	158.4 (5.7)
<i>P. alpina</i>	182.3 (13.6)	170.3 (7.3)	148.0 (19.4)
(b) leaf nitrogen content (mg·g ⁻¹)			
<i>C. foetida</i>	19.5 (0.9)	18.9 (1.1)	17.3 (0.9)
<i>A. gerardi</i>	16.8 (1.1)	17.7 (0.9)	16.9 (1.1)
<i>P. alpina</i>	14.8 (1.2)	17.3 (1.1)	14.0 (1.0)
(c) leaf dry matter content (mg·g ⁻¹)			
<i>C. foetida</i>	324.7 (5.2)	334.1 (2.9)	336.4 (3.4)
<i>A. gerardi</i>	303.6 (8.7)	296.7 (8.2)	316.7 (7.4)
<i>P. alpina</i>	326.1 (13.6)	310.1 (17.6)	345.1 (5.2)

radiative cooling (exposure to the cold night sky, leaf temperature < air temperature) brought plant temperatures close to their freezing points (about -10 °C; Fig. 5; Körner 1999). These findings are consistent with the results of Wipf *et al.* (2006, 2009), who found a negative correlation between the number of frost events (temperature < 0 °C) and vegetative growth of some subalpine species. During winter, graminoid leaf primordial tissues are buried a few centimeters belowground and are protected by dead leaf sheaths, and thus are not directly exposed to damaging frost events (Körner 1999); however, during spring, relatively high diurnal temperatures have the potential to activate growth despite very cold nights. The rise of primordia to the soil surface exposes them to freezing overnight temperatures and may lead to cell death, particularly since plants deharden rapidly when temperature and photoperiod increase (Bannister *et al.* 2005). Despite a capacity to recover very rapidly, the cost for the repair process might be too high to maintain cell function. Therefore, although the I treatment can be considered as extreme for snowbed communities, it underlines the potential sensitivity of snowbed species to frost events. Even if rare, such conditions might have long-term effects on the community composition of alpine snowbed tundra.

CONCLUSION

Although environmental conditions in this experiment differ between common garden and original habitat, the results highlight the importance of growth plasticity in determining species response to a lengthened growing season and in alleviating detrimental effects of early frost events on aboveground productivity in snowbed plant communities. In the context of climatic change, aperiodic species might be favoured compared to periodic species

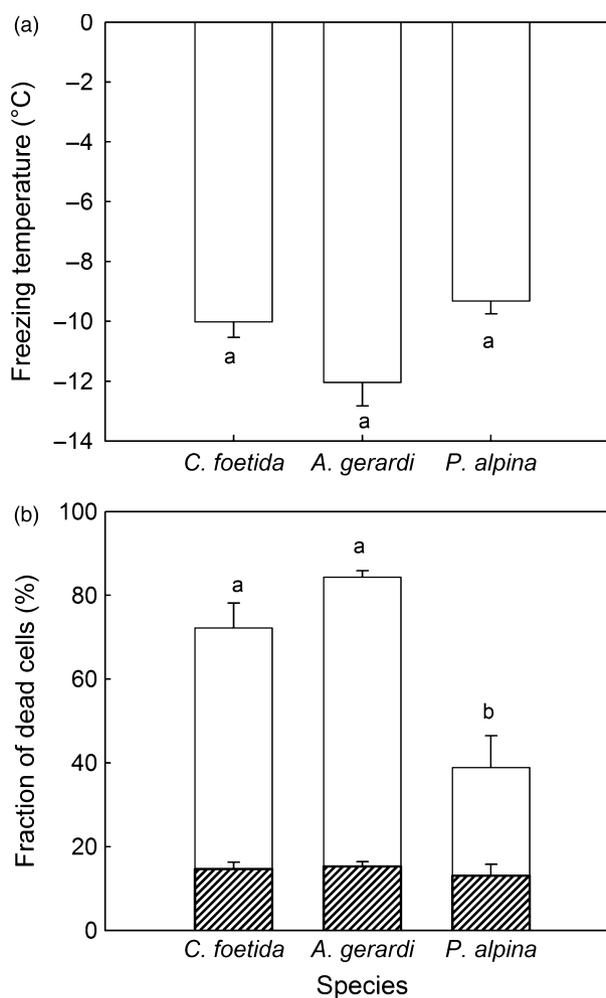


Fig. 5. Freezing temperature of new leaves (a) and electrolyte leakage in percentage (b) for *Carex foetida*, *Alopecurus gerardi* and *Poa alpina*. In b, higher (white bars) and lower values (hatched bars) correspond to samples measured after and before freezing treatment, respectively. Different letters indicate significant differences between species ($P < 0.05$). See main text for more details on statistical analysis.

as they might have greater scope to efficiently respond to an extended growing season. Larger comparative analyses of frost resistance and leaf phenology along snowmelt gradients in alpine tundra are necessary to assess in greater detail how these ecosystems will respond to climatic changes.

ACKNOWLEDGEMENTS

We are grateful to Serge Aubert, Cécile Bayle, Jean-Christophe Clément, Rolland Douzet, Geneviève Girard and Richard Hurstel for help in the field and in the laboratory. Logistical support was provided by the Station Alpine Joseph Fourier, the alpine field station of the

University Joseph Fourier. We thank Marie Corbel for her friendly logistical assistance, Matthew Robson, Fabien Quétier, five anonymous reviewers and the associate editor for critical comments and valuable insights on an earlier version of the manuscript. The project was financially supported by a grant from the French Research and Education Ministry and from the University Joseph Fourier of Grenoble.

SUPPORTING INFORMATION

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

Figure S1. Pictures of five monoliths from the E treatment taken on 17 May 2006 (a) and from L treatment taken on the 14 June 2007 (b) at the common garden of the Joseph Fourier Alpine Station (2100 m asl).

Figure S2. Soil water content (% vol) in I, L and E treatments from mid-May to early July 2007. Mean \pm SE, $n = 5$. Soil water availability was measured using time domain reflectometry (TDR probes, mini TRASE system 1, Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

Figure S3. Daily mean soil temperature (5-cm deep) for the I treatment (black) and air temperature (grey) in 2005–2006 (a) and in 2006–2007 (b).

Table S1. Mean snowmelt dates in snowbeds located in the Combe Roche Noire valley (2550 m asl) and close to the Galibier Pass (2600 m asl) from 2004 to 2007. Mean \pm SD ($n = 4$).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

REFERENCES

- Ball M.C., Wolfe J., Canny M., Hofmann M., Nicotra A.B., Hughes D. (2002) Space and time dependence of temperature and freezing in evergreen leaves. *Functional Plant Biology*, **29**, 1259–1272.
- Bannister P., Maegli T., Dickinson K.J.M., Halloy S.R.P., Knight A., Lord J.M., Mark A.F., Spencer K.L. (2005) Will loss of snow cover during climatic warming expose New Zealand alpine plants to increased frost damage? *Oecologia*, **144**, 245–256.
- Baptist F., Choler P. (2008) A simulation on the importance of growing season length and canopy functional properties on the seasonal Gross Primary Production of temperate alpine meadows. *Annals of Botany*, **101**, 549–559.
- Beniston M. (2005) Mountain climates and climatic change: an overview of processes focusing on the European Alps. *Pure and Applied Geophysics*, **162**, 1587–1606.
- Björk R.G., Molau U. (2007) Ecology of alpine snowbed and the impact of global change. *Arctic, Antarctic and Alpine Research*, **39**, 34–43.
- Bowman W.D. (1992) Inputs and storage of nitrogen in winter snowpack in an alpine ecosystem. *Arctic and Alpine Research*, **24**, 211–215.
- Brooks P.D., Williams M.W., Schmidt S.K. (1998) Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. *Biogeochemistry*, **43**, 1–15.
- Choler P. (2005) Consistent shifts in alpine plant traits along a mesotopographical gradient. *Arctic, Antarctic and Alpine Research*, **37**, 444–453.
- Cornelissen J.H.C., Lavorel S., Garnier E., Diaz S., Buchmann N., Gurvich D.E., Reich P.B., ter Steege H., Morgan H.D., van der Heijden M.G.A., Pausas J.G., Poorter H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335–380.
- Cunningham S.A., Summerhayes B., Westoby M. (1999) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecology*, **69**, 569–588.
- Dorrepaal E., Aerts R., Cornelissen J.H.C., Callaghan T.V., Van Logtestijn R.S.P. (2003) Summer warming and increased winter snow cover affect *Sphagnum fuscum* growth, structure and production in a sub-arctic bog. *Global Change Biology*, **10**, 93–104.
- Dunne J.A., Harte J., Taylor K.J. (2003) Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological Monographs*, **73**, 69–86.
- Dye D.G., Tucker C.J. (2003) Seasonality and trends of snow-cover, vegetation index, and temperature in northern Eurasia. *Geophysical Research Letters*, **30**, 9–12.
- Edwards A.C., Scalenghe R., Freppaz M. (2007) Changes in the seasonal snow cover of alpine regions and its effect on soil processes: a review. *Quaternary International*, **172**, 162–163.
- Galen C., Stanton M.L. (1993) Short-term responses of alpine buttercups to experimental manipulations of growing season length. *Ecology*, **75**, 1546–1557.
- Galen C., Stanton M.L. (1995) Responses of snowbed plant species to changes in growing-season length. *Ecology*, **76**, 415–436.
- Garnier E., Cortez J., Billes G., Navas M.L., Roumet C., Debussche M., Laurent G., Blanchard A., Aubry D., Bellmann A., Neill C., Toussaint J.P. (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, **85**, 2630–2637.
- Gianoli E. (2004) Plasticity of traits and correlations in two populations of *Convolvulus arvensis* (Convolvulaceae) differing in environmental heterogeneity. *International Journal of Plant Sciences*, **165**, 825–832.
- Hobbie S.E., Chapin F.S. (1996) Winter regulation of tundra litter carbon and nitrogen dynamics. *Biogeochemistry*, **35**, 327–338.
- Inouye D.W. (2000) The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters*, **3**, 457–463.

- Jaeger C.H., Monson R.K. (1992) Adaptive significance of nitrogen storage in *Bistorta bistortoides*, an alpine herb. *Oecologia*, **92**, 121–131.
- Jones M.H., Bay C., Nordenhäll U. (1997) Effects of experimental warming on arctic willows (*Salix* spp.): a comparison of responses from the Canadian High Arctic, Alaskan Arctic, and Swedish Subarctic. *Global Change Biology*, **3**, 55–60.
- Körner C. (1999) *Alpine Plant Life*. Springer Verlag, Berlin, Germany.
- Lipson D.A., Monson R.K., Bowman W.D. (1996) Luxury uptake and storage of nitrogen in the rhizomatous alpine herb, *Bistorta bistortoides*. *Ecology*, **77**, 569–576.
- Lipson D.A., Schmidt S.K., Monson R.K. (1999) Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology*, **80**, 1623–1631.
- Molau U. (1993) Relationships between flowering phenology and life history strategies in tundra plants. *Arctic and Alpine Research*, **25**, 391–402.
- Molau U. (1997) Responses to natural climatic variation and experimental warming in two tundra plant species with contrasting life forms: *Cassiope tetragona* and *Ranunculus nivalis*. *Global Change Biology*, **3**, 97–107.
- Molau U., Nordenhäll U., Eriksen B. (2005) Onset of flowering and climate variability in an alpine landscape: a 10-year study from Swedish Lapland. *American Journal of Botany*, **92**, 422–431.
- Monson R.K., Rosenstiel T.N., Forbis T.A., Lipson D.A., Jaeger C.H. (2006) Nitrogen and carbon storage in alpine plants. *Integrative and Comparative Biology*, **46**, 35–48.
- Neuner G., Pramsohler M. (2006) Freezing and high temperature thresholds of photosystem 2 compared to ice nucleation, frost and heat damage in evergreen subalpine plants. *Physiologia Plantarum*, **126**, 196–204.
- Nogués-Bravo D., Araujo M.B., Errea M.P., Martínez-Rica J.P. (2007) Exposure of global mountain systems to climate warming during the 21st Century. *Global Environmental Change*, **17**, 420–428.
- Price M.V., Waser N.M. (2000) Responses of subalpine meadow vegetation to four years of experimental warming. *Ecological Applications*, **10**, 811–823.
- Quétiér F., Thebault A., Lavorel S. (2007) Plant traits in a state and transition framework as markers of ecosystem response to land-use change. *Ecological Monographs*, **77**, 33–52.
- Saavedra F., Inouye D.W., Price M.V., Harte J. (2003) Changes in flowering and abundance of *Delphinium nuttallianum* (Ranunculaceae) in response to a subalpine climate warming experiment. *Global Change Biology*, **9**, 885–894.
- Schöb C., Kammer P.M., Choler P., Veit H. (2009) Small-scale plant species distribution in snowbeds and its sensitivity to climate change. *Plant Ecology*, **200**, 91–104.
- Scott P.A., Rouse W.R. (1995) Impacts of increased winter snow cover on upland tundra vegetation: a case example. *Climate Research*, **5**, 25–30.
- Serreze M.C., Walsh J.E., Chapin F.S., Osterkamp T., Dyurgerov M., Romanovsky V., Oechel W.C., Morison J., Zhang T., Barry R.G. (2000) Observational evidence of recent change in the northern high-latitude environment. *Climatic Change*, **46**, 159–207.
- Shaver G.R., Laundre J. (1997) Exsertion, elongation, and senescence of leaves of *Eriophorum vaginatum* and *Carex bigelowii* in Northern Alaska. *Global Change Biology*, **3**, 146–157.
- Sørensen T. (1941) Temperature relations and phenology of the northeast Greenland flowering plants. *Meddelelser om Grønland*, **125**, 1–305.
- Starr G., Oberbauer S.F., Pop E.W. (2000) Effects of lengthened growing season and soil warming on the phenology and physiology of *Polygonum bistorta*. *Global Change Biology*, **6**, 357–369.
- Stenström A., Jónsdóttir I.S. (1997) Responses of the clonal sedge, *Carex bigelowii*, to two seasons of simulated climate change. *Global Change Biology*, **3**, 89–96.
- Stenström M., Gugerli F., Henry G.H.R. (1997) Response of *Saxifraga oppositifolia* L. to simulated climate change at three contrasting latitudes. *Global Change Biology*, **3**, 44–54.
- Taschler D., Neuner G. (2004) Summer frost resistance and freezing patterns measured *in situ* in leaves of major alpine plant growth forms in relation to their upper distribution boundary. *Plant, Cell and Environment*, **27**, 737–746.
- Theurillat J.P., Guisan A. (2001) Potential impact of climate change on vegetation in the European Alps: a review. *Climatic Change*, **50**, 77–109.
- Tomaselli M. (1991) The snow-bed vegetation in the Northern Apennines. *Vegetation*, **94**, 349–354.
- de Valpine P., Harte J. (2001) Plant responses to experimental warming in a montane meadow. *Ecology*, **82**, 637–648.
- Wahren C.H.A., Walker M.D., Bret-Harte M.S. (2005) Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. *Global Change Biology*, **11**, 537–552.
- Webb M.S., Gilmour S.J., Thomashow M.F., Steponkus P.L. (1996) Effects of COR6.6 and COR15am polypeptides encoded by COR (cold-regulated) genes of *Arabidopsis thaliana* on dehydration-induced phase transitions of phospholipid membranes. *Plant Physiology*, **111**, 301–312.
- Welker J.M., Molau U., Parsons A.N., Robinson C.H., Wookey P.A. (1997) Responses of *Dryas octopetala* to ITEX environmental manipulations: a synthesis with circumpolar comparisons. *Global Change Biology*, **3**, 61–73.
- Wipf S., Rixen C., Mulder C.P.H. (2006) Advanced snowmelt causes a shift towards positive neighbour interactions in a subarctic tundra community. *Global Change Biology*, **12**, 1496–1506.
- Wipf S., Stoeckli V., Bebi P. (2009) Winter climate change in alpine tundra: plant responses to changes in snow depth and snowmelt timing. *Climatic Change*, **94**, 105–121.
- Wright I.J., Reich P.B., Westoby M. (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology*, **15**, 423–434.