

The snow and the willows: earlier spring snowmelt reduces performance in the low-lying alpine shrub *Salix herbacea*

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Summary

1. Current changes in shrub abundance in alpine and arctic tundra ecosystems are primarily driven by climate change. However, while taller shrub communities are expanding, dwarf shrub communities show reductions under climate warming, and the mechanisms driving the latter (such as warming temperatures or accelerated spring snowmelt) may be complex.

2. To determine and disentangle the response of a widespread arctic–alpine prostrate dwarf shrub to both climate warming and changes in snowmelt time, we investigated phenology, clonal and sexual reproduction, leaf size, wood tissue carbon balance and leaf damage in 480 patches of *Salix herbacea*, along its elevational and snowmelt microhabitat range over 3 years in a space-for-time substitution.

3. Earlier snowmelt was associated with longer phenological development periods, an increased likelihood of herbivory and fungal damage, lower stem density, smaller leaves and lower end-of-season wood reserve carbohydrates. Furthermore, while early snowmelt was associated with an increased proportion of flowering stems, the proportion of fruiting stems was not, as fruit set decreased significantly with earlier snowmelt. Warmer temperatures at lower elevations were associated with lower stem numbers and larger leaves.

4. *Synthesis.* Our study indicates that phenology, fitness proxies and fungal/insect damage of the dwarf shrub *S. herbacea* are strongly influenced by snowmelt timing, and that earlier spring snowmelt reduced performance in *S. herbacea*. The likely mechanisms for many of the observed patterns are related to adverse temperature conditions in the early growing season. Reductions in clonal (stem number) and sexual reproduction (reduced fruit set) under earlier snowmelt, in addition to increasing damage probability, will likely lead to lower fitness and poorer performance, particularly in shrubs growing in early-exposure microhabitats. Further, we saw few concurrent benefits of higher temperatures for *S. herbacea*, particularly as warming was associated with lower clonal growth. As growing seasons become warmer and longer in arctic and alpine tundra ecosystems, early snowmelt is a critical mechanism reducing fitness and performance in a widespread dwarf shrub and may ultimately reduce dwarf shrub communities in tundra biomes.

Key-words: climate change, clonal reproduction, dwarf shrub, early snowmelt, flowering, herbivory, non-structural carbohydrates, phenology, plant–climate interactions

Introduction

Shrub encroachment, the expansion and increased growth of shrub communities as a response to climate change, is occurring at an unprecedented rate in many tundra ecosystems (e.g.

Myers-Smith *et al.* 2011; Elmendorf *et al.* 2012). Shrub expansion may create multiple feedbacks, through snow trapping, warming via albedo alteration and alterations of soil temperature and moisture (e.g. Sturm *et al.* 2005; Myers-Smith *et al.* 2011). However, shrub encroachment is mainly driven by the expansion of taller shrubs (Elmendorf *et al.* 2012); in contrast, dwarf shrubs (<15 cm in height) show widespread reductions as a response to warming (Elmendorf

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et al. 2012), with simulations showing up to 60% habitat loss for prostrate dwarf shrub tundra (Kaplan & New 2006). Since they represent such a major component of groundcover in arctic and alpine tundra, it is important to understand both the effects of climate change on dwarf shrubs and their capacity to respond to environmental change. Due to their low stature (and often prostrate growth forms), many dwarf shrubs are strongly ecologically and phenologically dependent on snowmelt timing (Wipf, Stoeckli & Bebi 2009; Wipf & Rixen 2010), so it is critical to consider responses to both warming and spring snowmelt time. While many recent studies have focussed on the effects of either warming or altered snowmelt on taller deciduous dwarf shrubs (e.g. Wipf 2010; Rixen *et al.* 2012; Gerdol *et al.* 2013; Anadon-Rosell *et al.* 2014), the responses of prostrate shrubs, particularly deciduous species, to warming and snowmelt change have been relatively understudied (but see Clemmensen & Michelsen 2006; Kudo *et al.* 2010), and rarely so for both factors in combination.

Predicting dwarf shrub response to warming and snowmelt change, however, can be difficult. Prostrate shrub forms may be strongly decoupled from the atmospheric temperatures (Körner 2003b), and thus may be less responsive to temperature extremes. Further, arctic and alpine dwarf shrubs vary in their responses to warming and early snowmelt, and different traits do not respond in concert, even within species. Warming has been shown to increase growth and biomass production in the dwarf shrub *Cassiope tetragona*, but not in co-occurring prostrate *Salix arctica* (Campioli *et al.* 2013). The low-lying alpine shrubs *Arctostaphylos alpina* and *Vaccinium uliginosum* have demonstrated increased flower production under warming, but no increase in seedling establishment (Suzuki & Kudo 2005). Earlier snowmelt has been demonstrated to benefit vegetative growth in *Loiseleuria procumbens* but not in three other co-occurring dwarf shrubs (*Empetrum nigrum*, *V. uliginosum*, *Vaccinium myrtillus*; Wipf, Stoeckli & Bebi 2009). Early snowmelt also accelerates phenology, but with no corresponding vegetative growth or reproductive response in eight tundra dwarf shrubs (Wipf 2010). In addition, potentially important traits, such as seasonal carbon accumulation, have been extensively studied and have contributed to understanding growth limitation in trees at tree line (e.g. Körner 2003a; Hoch & Körner 2012) but have rarely if ever been examined in low alpine shrubs. Studies examining shrub responses to global change typically focus only on a narrow range of traits (e.g. relative growth rate, flowering, but see Wipf, Stoeckli & Bebi 2009; Wipf 2010), which may not be able to capture all important responses of typically long-lived, clonal tundra shrubs.

Snowmelt timing and warming may also alter the multi-trophic biotic interactions for the whole shrub community, particularly as it affects exposure to herbivores and pathogens (Roy, Gusewell & Harte 2004). Again, predicting dwarf shrub responses is difficult, as later-melting snow cover may shelter low-lying shrubs by acting as a barrier to herbivores and pathogens or serve to intensify later herbivory damage to shrub leaf tissue (Roy, Gusewell & Harte 2004). Timing of snowmelt will also control the temperature regime, as acceler-

ated snowmelt will allow shrubs to accumulate greater temperature sums over the course of the growing season, but also increases exposure to freezing damage (Inouye 2008; Wheeler *et al.* 2014).

Salix herbacea L. is a dioecious, widespread and often dominant prostrate dwarf shrub common in arctic and alpine tundra, and represents one species facing both warming climates and earlier snowmelt. It is an ideal species for studying the effects of both warming and snowmelt timing, as it occurs along a relatively long elevational gradient and occupies a wide range of microsite types, from rocky, early-exposure ridges to late-season snowbeds (Beerling 1998). Further, in our study area, we did not detect any neutral genetic differentiation indicating past or present restrictions of gene flow between subpopulations of *S. herbacea* in early and late snowmelt microhabitats, or along elevational gradients, suggesting rampant gene flow throughout the entire *S. herbacea* population (Cortés *et al.* 2014). This makes it more likely that environmental differences rather than genetic differentiation govern trait differences between subpopulations along snowmelt or elevational gradients, although genetic differentiation at loci under strong local selection cannot be ruled out (Cortés *et al.* 2014). In our study site in the eastern Swiss Alps, elevation and snowmelt timing are generally decoupled (Wheeler *et al.* 2014), likely due to the highly heterogenous microtopography characteristic to alpine terrain (Scherrer & Körner 2011). This allows us to consider elevation and snowmelt as independent drivers of prostrate shrub responses to both warming (at lower elevation) and earlier snowmelt time.

Our study seeks to disentangle and determine the effects of two global change drivers, accelerated spring snowmelt and warmer growing season temperatures, on a comprehensive range of *S. herbacea* traits and fitness proxies, and whether both sexes respond similarly along these environmental gradients. To this end, we collected a large data set over an extended time frame: we investigated 480 *S. herbacea* shrubs in the field, along their elevational and snowmelt microhabitat range over three consecutive growing seasons in a space-for-time substitution. We used the temperature changes along the elevational gradient to represent shifts in mean temperature, similar to warming temperatures under climate change, and microhabitat differences were used to simulate earlier snowmelt. We investigated a wide range of functional traits along these gradients, and as a function of sex: we examined phenophase timing and developmental period, flower and fruit production, vegetative growth, carbon balance [accumulation of non-structural carbohydrates (NSCs) during the growing season] and the likelihood of leaf damage by insect herbivores and fungal pathogens. The objectives of this study were to determine how each set of traits responded to (i) earlier snowmelt timing and thus an elongated growing season and (ii) warmer growing season temperatures at lower elevations, in order to evaluate performance in a widespread prostrate shrub under climate change scenarios. We hypothesize earlier snowmelt will accelerate phenological timing, but will not benefit vegetative growth or sexual reproduction at higher elevation,

while warming at lower elevations will increase sexual reproduction and vegetative production. This study thus represents a novel use of a space-for-time substitution to predict the effects of two global change drivers on an extensive range of fitness proxies, and phenological, growth and carbon balance traits, in addition to probability of damage by herbivores and pathogens. The results of this study will indicate the mechanisms driving trait differences, which will help predict potential fitness responses and shifts in the low-lying shrub community in alpine and arctic ecosystems in response to climate change.

Materials and methods

STUDY SPECIES AND SITES

Our study took place along three elevational transects established on mountains near Davos, in the eastern Swiss Alps: Jakobshorn (46°46' N, 09°50' E, 2100–2600 m a.s.l.), Schwarzhorn (46°44' N; 09°57' E, 2380–2780 m a.s.l.) and Wannengrat (46°48' N, 09°46' E, 2280–2640 m a.s.l.). All transects had similar NE exposure and covered the main elevational range of *S. herbacea*. At 2100 m a.s.l. at Jakobshorn, a site generally representative of the region, 1975–2012, average annual air temperature is 2.06 °C and annual precipitation is 1150 mm, with snowfall occurring in all months of the year (Rixen *et al.* 2012). During this 37-year measurement period, air temperatures during the warmest months (June–August) have increased by 0.58 °C per decade, and spring snowmelt has advanced by 3.5 days per decade (Rixen *et al.* 2012).

DATA COLLECTION

At 10 elevational bands along transects on each of the three mountains, we established study plots (approximately 3 × 3 m) in two ridge microhabitat sites (early-season exposure from snow) and two late snowbed microhabitat sites (late-season exposure), for a total of 120 plots. Plots were established as closely together as feasible, given the ground conditions, and were situated between <5 up to approximately 20 m apart. In each plot, we selected four *S. herbacea* patches of 10 cm diameter, for a total of 480 patches, and marked them for monitoring. Of the marked patches, 65 were identified as male, 227 were female and the remainder did not flower during the three seasons of this study. Sex ratios were not significantly different along the elevational or snowmelt gradients. All *S. herbacea* patches were spaced at least 1 m apart, to reduce the probability of sampling the same clone multiple times (Stamati, Hollingsworth & Russell 2007).

We monitored patches weekly from snowmelt until leaf senescence during the 2011, 2012 and 2013 growing seasons. For each patch, we recorded snowmelt timing, leaf developmental status (bud break, rolled, open), proportion of stems flowering and proportion of stems fruiting (newly mature fruit) weekly and used these data to determine first observation date of each phenophase (phenological stages: leaf opening, flowering and fruiting) and fruit set (rate of successful transition from flowers to fruit). We also recorded leaf tissue damage, with proportion of patch leaves damaged by herbivores and fungi since these two were conclusive damage agents. Leaf browning, potentially as a result of frost damage, was also observed, but not included in later analysis as the actual cause of damage could not be conclusively determined.

For each patch, we recorded stem density (i.e. number of stems per 10-cm diameter) and mean leaf area (i.e. the average area per leaf of two leaves) after full leaf expansion. As below-ground biomass calculations were impossible in *S. herbacea* due to complexity of the underground root system, the extensive overlapping of clones and the destructive nature of such sampling, we used stem number as a proxy for all biomass production. In support of this argument, a glasshouse pot experiment showed above-ground stem production correlated strongly with below-ground biomass production ($r = 0.86$, $P < 0.001$; J. Sedlacek, unpubl. data).

We performed NSC analysis on sampled wood tissue (stems) at the end of the 2012 field season (sampling performed on 16-09-2012). Sampled material was dried at 75 °C in a drying-oven for 72 h within a maximum of 6 h after sampling, ground to fine powder in a ball mill and stored dry over silica gel until analysis. NSC were analysed using the protocol for the enzymatic–photometric analysis detailed by Hoch, Popp & Körner (2002). NSC was considered to be the sum of the three quantitatively most important free low molecular weight sugars (glucose, fructose and sucrose) plus starch, and are expressed as percentage of dry wood mass.

We recorded soil temperature (5 cm below the surface, at 2-h intervals) and snowmelt date for each plot using *in-situ* soil temperature loggers (iButton; Maxim Integrated, San Jose, CA, USA). Logger data were used in conjunction with field observations to determine day of snowmelt (date when soil temperature rose abruptly from the near-0 °C that characterizes continued snow cover; Rixen *et al.* 2012). We calculated growing degree days (GDD) above 5 °C as derived from the 2-h interval means; GDD accumulation was calculated from snowmelt to the end of August for each site and from snowmelt to the first appearance of each phenophase. We used a climate station at the base of the Jakobshorn transect (2100 m a.s.l.) to extrapolate the frequency of frost events (daily air temperature minima below 0 °C) for each microhabitat plot during the 2011 and 2012 growing season. The number of subzero temperature events during the snow-free period at each plot along the elevational and snowmelt gradients were then extrapolated from the daily air temperature minima at the climate station using an elevational lapse rate of 0.52 K 100 m⁻¹, an average lapse rate for the eastern Swiss Alps (Kollas 2013; Wheeler *et al.* 2014).

DATA ANALYSIS

Links between elevation and snowmelt time were analysed using a Pearson's correlation, with data for all years pooled. Mean soil temperatures during the growing season were analysed along the elevational and snowmelt gradients using a general linear mixed model. Fixed terms were snowmelt day and elevation, both centred to a mean of zero to allow appropriate effect estimation (Schielzeth 2010). Snowmelt day and elevation were chosen as the fixed terms for the models as mean growing season soil temperature was highly correlated with both elevation and snowmelt day, while elevation and snowmelt day were not statistically significantly correlated. Thus, we selected elevation to act as a general proxy for growing season temperature effects. The nested random factors were transect, elevational band (an alphanumeric category indicating the proximity of four study plots per elevational band), study plot and individual patch nested within study plot, with year as an additional crossed random effect.

Variation in all traits was analysed using generalized linear mixed models. Onset of phenophases and phenological development times to leaf expansion, flowering and fruiting were all highly correlated, so

for simplicity we report only onset of and development time to flowering. For models examining phenological development time, wood NSC, wood starch, wood sugar and leaf area, we used a normal error distribution. If necessary, response variables were transformed to ensure normality of residuals, and diagnostic plots of residuals were used when appropriate to confirm a good model fit and normal distribution of the residuals. For models examining proportion of patch flowering and fruiting, probability of fruit set and the presence/absence of leaf damage (from herbivory and fungi), we used a binomial error distribution, and for stem density we used a Poisson error distribution. The fixed terms in all models were snowmelt day, elevation (both centred to a mean of zero; Schielzeth 2010) and sex, for traits not related to fruiting. The two-way interactions of all fixed terms were included in all initial models, then dropped if the interaction was non-significant and did not improve model fit. The nested random factors were plant patch, study plot, elevational band and transect, with year as an additional crossed random effect. Year was included as a random effect since preliminary analysis indicated that response trends were generally similar between years. To ensure the number of parameters tested did not unduly influence the results, all *P*-values of responses to snowmelt time, elevation and sex effects were compared to values generated using the Benjamini and Hochberg False Discovery Rate (Verhoeven, Simonsen & McIntyre 2005). We also ran the models using linear and general linear models to examine mean response values across plot and year, which produced generally similar results to our mixed models. All statistical analyses were carried out in R v.3.1.2, using packages lme4 (Bates *et al.* 2014) and lmerTest (Kuznetsova, Brockhoff & Christensen 2015).

Results

TEMPERATURE AND SNOWMELT ALONG ENVIRONMENTAL GRADIENTS

There was no significant correlation between elevation and snowmelt time in all years combined ($r = 0.051$, $t = 1.55$, $P = 0.12$), suggesting that localized microhabitat effects are more important drivers of snowmelt timing compared to landscape-scale elevational gradients and the associated adiabatic air temperature change with altitude. Growing seasons were defined separately for each plot, depending on local snowmelt timing. Mean soil temperatures during both the full growing season in each plot (i.e. from date of snowmelt to the end of August) and in the first 30 days following snowmelt increased significantly with later snowmelt (est = 0.45, NumDF = 1, DenDF = 78.09, $t = 3.58$, $P < 0.001$; est = 1.67, NumDF = 1, DenDF = 45.93, $t = 6.78$, $P < 0.001$; Fig. 1). Along the elevational gradient, full growing season mean temperatures decreased significantly with higher elevation, while temperature in the first 30 days was not significantly affected (est = -0.69, NumDF = 1, DenDF = 29.76, $t = -3.56$, $P = 0.0013$; est = -0.49, NumDF = 1, DenDF = 63.00, $t = -1.82$, $P = 0.074$; Fig. 1). There was a significant interaction effect between snowmelt time and elevation on mean soil temperature in the first 30 days after snowmelt (est = -0.63, NumDF = 1, DenDF = 61.33, $t = -2.12$, $P = 0.039$) and a marginally significant interaction effect of snowmelt time and elevation on mean soil temperature for the whole growing season

(est = -0.27, NumDF = 1, DenDF = 95.26, $t = -1.92$, $P = 0.058$).

The frequency of frost events in the growing season was extrapolated to be significantly more frequent both with increasing elevation (2011: est = 0.27, DF = 35, $z = 6.45$, $P < 0.001$; 2012: est = 0.33, DF = 34, $z = 4.45$, $P < 0.001$) and with earlier snowmelt timing (2011: est = -0.25, DF = 36, $z = -6.40$, $P < 0.001$; 2012: est = -0.41, DF = 35, $z = -6.39$, $P < 0.001$; Fig. S1 in Supporting Information).

PHENOLOGY AND DEVELOPMENTAL TIMING

The onset of flowering ($N = 738$) was controlled by snowmelt day, with significantly later flowering onset with later snowmelt (Table 1 and Fig. 2). The developmental period (time from snowmelt to flowering) was, however, significantly shorter than later snowmelt (Table 1 and Fig. 2). Elevation did not significantly influence onset of or development time to flowering (Table 1).

Temperatures accumulated at the onset of flowering (i.e. GDD from snowmelt to flowering) were not significantly different along elevation or snowmelt gradients (flowering GDD along elevation gradient: $t = 0.19$, $P = 0.84$; along snowmelt gradient: $t = -1.12$, $P = 0.26$), suggesting that similar temperature-accumulation thresholds must be reached for phenological development along the species range, at least for the early phenophases.

REPRODUCTION

The proportion of stems flowering decreased significantly with later snowmelt ($N = 691$; Table 1 and Fig. 3). The proportion of stems fruiting, however, was similar along the snowmelt gradient ($N = 406$; Table 1 and Fig. 2). Elevation had no significant effect on flowering or fruiting proportion, sex had no effect on flowering and there were no significant interaction effects. The difference between flower and fruit production along the snowmelt gradient was explained by fruit set, which increased significantly with later snowmelt (Table 1 and Fig. 3). Hence, the resource allocation to higher flowering proportions under early snowmelt did not pay off for the plant in terms of actual fruit set. There were no significant interaction effects between elevation and snowmelt timing for flower or fruit production, or for fruit set (Table 1). There was no significant difference in flowering proportion between male and female plants, and no significant interaction effects between sex and elevation or snowmelt (Table 1).

Frost events significantly impacted fruit production, with patch fruiting proportion decreasing significantly with increasing frequency of frost events during the growing season (numDF = 1, denDF = 115, $t = -2.57$, $P = 0.011$; Fig. S2).

VEGETATIVE GROWTH

Mean area per leaf ($N = 324$) was primarily controlled by elevation, with leaf size decreasing as elevation increased, and to

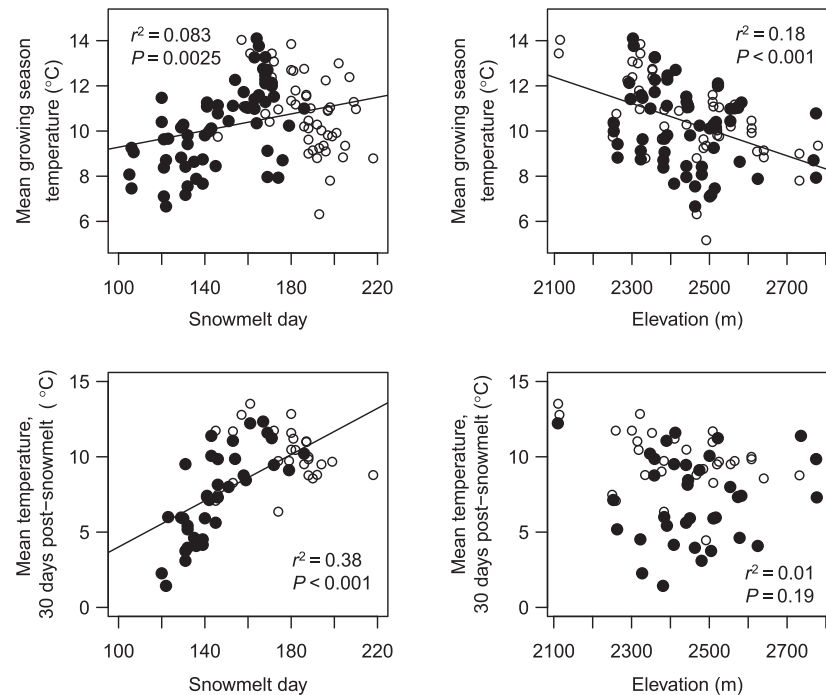


Fig. 1. Mean soil temperature at a depth of 5 cm during the growing season (day of snowmelt until the end of August) along the snowmelt gradient (upper left) and elevation gradient (upper right), and mean soil temperature during the first 30 days after snowmelt along the snowmelt gradient (lower left) and elevation gradient (lower right) over 2 years on three mountains in Davos, Switzerland. Black points represent data from ridge microhabitats, and white points represent snowbeds. Data are fitted with a regression curve when the relationship is statistically significant.

a lesser extent by snowmelt timing, with leaf size increasing with later snowmelt. Females had slightly larger leaves than males (female mean leaf size = 82.1 mm², SE = 1.68; male mean leaf size = 76.2 mm², SE = 2.46; Table 1). Shrub stem density did not differ significantly between the sexes and increased with both elevation and later snowmelt ($N = 333$; Table 1 and Fig. 4). In addition, there was a significant interaction between sex and elevation, with male stem density increasing more rapidly with elevation (Table 1 and Fig. 4).

NON-STRUCTURAL CARBOHYDRATE CONCENTRATIONS

Late-season wood tissue NSC and starch concentrations in stem wood were relatively high overall (NSC mean = 21.7% dry mass, SE = 0.3; starch mean = 14.7% dry mass, SE = 0.2), demonstrating that carbon was likely not limiting across the entire gradient. Both NSC and low molecular weight sugars increased significantly with later snowmelt ($N = 154$; Table 1). There were no significant sex differences between NSC, starch or low molecular weight sugars concentrations, nor were there significant interaction effects between sex, snowmelt time or elevation (Table 1).

LEAF DAMAGE LIKELIHOOD

Herbivory and fungal damage to leaf tissue were both relatively common, with herbivory occurring in 43.6% of the patches and fungal damage occurring in 40.2%. The probability of leaf damage from herbivores and fungi decreased significantly with later snowmelt ($N = 777$; Table 1, Fig. S3). There was no significant effect of elevation, sex or any interaction effects on the probability of leaf herbivory or fungal damage (Table 1).

Discussion

In this study, variations in phenological, growth and reproductive traits, and probability of damage by herbivores and fungi in *S. herbacea* were strongly associated with snowmelt timing. Sex did not strongly affect responses in this dioecious species. Our results suggest that earlier spring snowmelt time, with associated low-temperature events during the growing season, generally reduces performance in *S. herbacea*. The poorer growth and reproductive traits under earlier snowmelt, in addition to increasing damage probability, suggests a potential reduction in fitness in the dwarf shrub *S. herbacea* as a result of global change.

EARLIER BUT SLOWER PHENOLOGICAL DEVELOPMENT UNDER EARLY SNOWMELT

In arctic and alpine plant species, accumulation of temperature past a certain threshold is often a prerequisite for phenological transitions (Kudo & Suzuki 1999; Molau, Nordenhall & Eriksen 2005; Huelber *et al.* 2006). This is generally supported by our data, where GDD accumulated at the onset of flowering were similar along the snowmelt gradient and along the elevational gradient. Thus, we conclude temperature largely regulates phenological development within the studied environmental range of *S. herbacea*, as opposed to other potential drivers such as photoperiodism. It is unlikely that photoperiodism is a driver in our system, as day length at snowmelt is already near its annual maximum at our investigated transects, and a 15-h day length threshold is generally assumed for the onset of alpine plant growth (Heide 2001). Lower temperatures directly after snowmelt for early-exposed ridges imply that more time is required to reach each temperature-accumulation threshold

Table 1. Responses of *Salix herbacea* functional traits to elevation, snowmelt time, sex and the two-way interactions between these factors over 3 years on three mountains in Davos, Switzerland

Response trait	Transformation	Elevation	Snowmelt	Sex	Elevation*snow	Sex*elevation	Sex*snow
Day of flowering	Sqrt	Est = 0.025 nDF = 1 dDF = 57.9 $t = 1.10$ $P = 0.27$	Est = 0.61 nDF = 1 dDF = 510.6 $t = 21.03$ $P < 0.001^*$	Est = -0.030 nDF = 1 dDF = 689.1 $t = -0.97$ $P = 0.33$	None	None	Est = -0.066 nDF = 1 dDF = 740.4 $t = -2.36$ $P = 0.019$
Time from snowmelt to flowering	Sqrt	Est = 0.067 nDF = 1 dDF = 65.0 $t = 1.04$ $P = 0.31$	Est = -0.96 nDF = 1 dDF = 507.7 $t = -11.15$ $P < 0.001^*$	Est = -0.12 nDF = 1 dDF = 669.2 $t = -1.34$ $P = 0.18$	None	None	Est = -0.20 nDF = 1 dDF = 741.2 $t = -2.33$ $P = 0.020$
Proportion of patch flowering	NA	Est = -0.21 $z = -1.81$ $P = 0.34$	Est = -0.35 $z = -2.93$ $P = 0.0034^*$	Est = -0.24 $z = -0.94$ $P = 0.35$	None	None	None
Proportion of patch fruiting	NA	Est = -0.37 $z = -1.85$ $P = 0.065$	Est = 0.31 $z = 1.61$ $P = 0.11$	NA	None	NA	NA
Per cent fruit set	NA	Est = -0.13 $z = -1.22$ $P = 0.22$	Est = 0.50 $z = 4.60$ $P < 0.001^*$	NA	None	NA	NA
Mean leaf size	Log	Est = -0.13 nDF = 1 dDF = 96.3 $t = -4.99$ $P < 0.001^*$	Est = 0.062 nDF = 1 DF = 250.9 $t = 2.58$ $P = 0.010^*$	Est = 0.11 nDF = 1 DF = 306.8 $t = 2.45$ $P = 0.015$	None	None	None
Stem density	NA	Est = 0.34 $z = 5.80$ $P < 0.001^*$	Est = 0.10 $z = 3.82$ $P < 0.001^*$	Est = 0.041 $z = 0.75$ $P = 0.45$	None	Est = -0.18 $z = -3.51$ $P < 0.001^*$	None
Wood non-structural carbohydrate	None	Est = 0.40 nDF = 1 dDF = 46.2 $t = 1.65$ $P = 0.11$	Est = 0.85 nDF = 1 dDF = 146.8 $t = 3.32$ $P = 0.0011^*$	Est = -0.12 nDF = 1 dDF = 153.9 $t = -0.20$ $P = 0.85$	None	None	None
Wood starch	None	Est = -0.14 nDF = 1 dDF = 22.5 $t = -0.48$ $P = 0.64$	Est = 0.46 nDF = 1 dDF = 82.4 $t = 1.73$ $P = 0.086$	Est = -0.22 nDF = 1 dDF = 152.44 $t = -0.39$ $P = 0.69$	None	None	None
Wood low MW sugar	None	Est = 0.56 nDF = 1 dDF = 25.6 $t = 3.14$ $P = 0.0042^*$	Est = 0.28 nDF = 1 dDF = 126.1 $t = 2.04$ $P = 0.044$	Est = 0.28 nDF = 1 dDF = 146.3 $t = 1.05$ $P = 0.29$	None	None	None
Probability of leaf herbivory	NA	Est = 0.093 $z = 0.65$ $P = 0.51$	Est = -0.44 $z = -2.86$ $P = 0.0043^*$	Est = -0.24 $z = -0.92$ $P = 0.36$	None	None	None
Probability of leaf fungi	NA	Est = -0.13 $z = -1.07$ $P = 0.28$	Est = -0.26 $z = -2.47$ $P = 0.014^*$	Est = 0.083 $z = 0.39$ $P = 0.69$	None	None	None

The data are from general linear mixed models. Response trait transformation is listed as square root (sqrt), log or none/NA, with estimate (est), numerator and denominator degrees of freedom (numDF/denDF), t - or z -values and P -values (bolded when significant) from the mixed models.

*When significant P -values fell below Benjamini and Hochberg False Discovery Rate thresholds.

needed for phenological development, as early-season temperature accumulation is likely controlled by snowmelt time. This was supported by our results, where post-snowmelt temperatures were lower and phenological development was slower with earlier snowmelt. Early-exposure sites are further characterized by an increasing risk of damaging freezing events occurring after snowmelt (Inouye 2008; Wipf, Stoeckli & Bebi 2009). In our study sites, frost events were more frequent on

earlier snowmelt sites. Further, longer phenological development times may have fitness implications: Sedlacek *et al.* (2015) demonstrated reduced flowering in *S. herbacea* with longer phenological development times on early-snowmelt microhabitats. Consequently, the net outcome of earlier snowmelt for *S. herbacea* phenology is likely negative, as more time is spent in earlier phenological stages, when plant vulnerability to damage, particularly freezing, is higher (Lenz *et al.* 2013).

Fig. 2. Onset of flowering, and development time required from snowmelt to flowering for *Salix herbacea* averaged for both plot and year along the snowmelt gradient over 3 years on three mountains in Davos, Switzerland. Black points represent data from ridge microhabitats, and white points represent snowbeds. Data are fitted with a quadratic curve in order to show untransformed data.

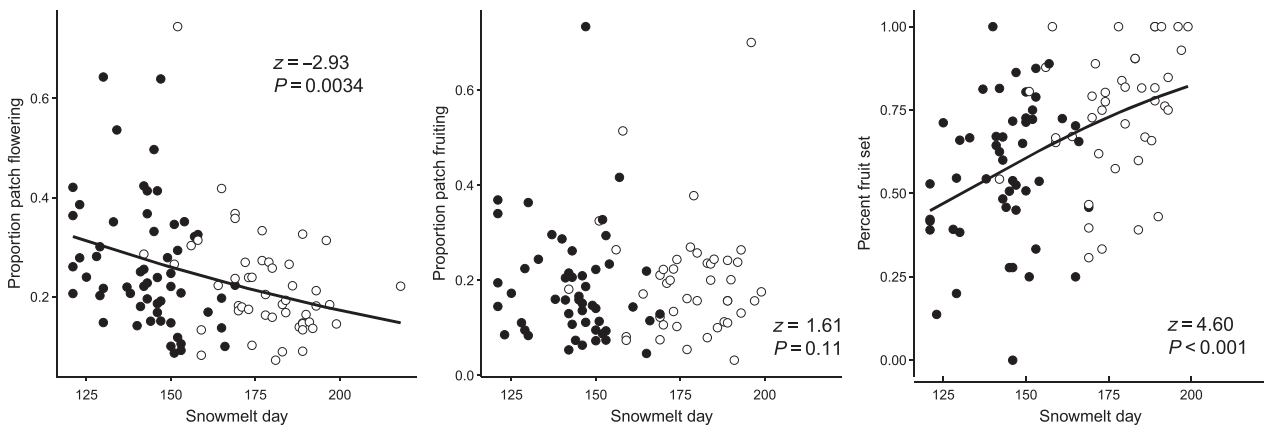
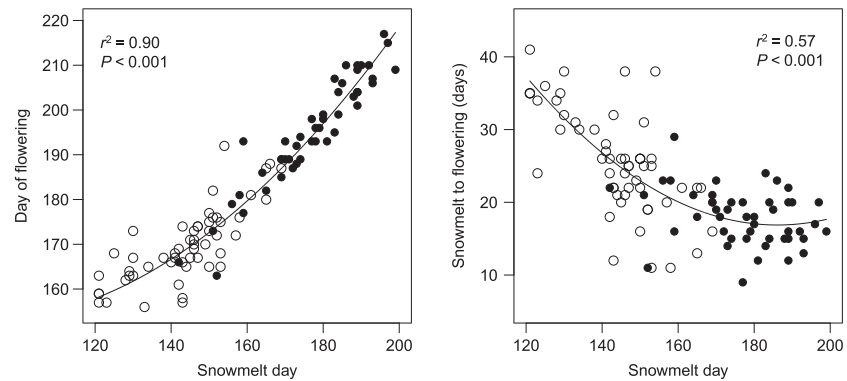


Fig. 3. Proportion of stems flowering and fruiting for *Salix herbacea*, and per cent fruit set averaged for both plot and year along the snowmelt gradient over 3 years on three mountains in Davos, Switzerland. Black points represent data from ridge microhabitats, while white points represent snowbeds. Data are fitted with a binomial regression curve when the relationship is statistically significant.

REDUCED FRUIT PRODUCTION AND VEGETATIVE GROWTH UNDER EARLY SNOWMELT

Localized snowmelt time and associated environmental conditions may play the most important role in determining the sexual reproductive response of *S. herbacea* to environmental change, as it controls fruit set. Female shrubs appear to increase flower production when exposed earlier from snow, but with no corresponding increase in fruiting success. Montesinos, Garcia-Fayos & Verdu (2012) also found a similar strong reproductive investment in female flowers in the dioecious shrub *Juniperus thurifera*, which did not lead to a high seed set, suggesting instead that drought, or extreme temperatures, control investment in fruit and seed production. In our study, the decreased success in fruit set with earlier snowmelt led to no changes in fruit produced along the snowmelt gradient, which indicates a loss of flowers on earlier snowmelt sites prior to fruit maturation. This could be explained by a greater likelihood of critical damage to reproductive structures on earlier snowmelt sites. We found a reduction in fruit production with increasing frequency of frost events, which were more common on earlier snowmelt sites. Loss of reproductive structures to episodic cold events may be common: Ladinig *et al.* (2013) found poor freezing resistance of reproductive

structures compared to vegetative structures in a number of alpine plants, and *S. herbacea* is likely frequently exposed to subzero temperatures on early snowmelt and high-elevation sites. Early-season freezing events could explain abortion rate of flowers on ridge shrubs and lack of corresponding fruit increase on earlier snowmelt sites. In addition, reduced fruit set in earlier snowmelt sites could be related to pollen limitation, potentially caused by fewer insect pollinators or co-flowering males; in a pollen addition experiment, Nielsen (2014) found that natural pollen limitation limited seed set in *S. herbacea*.

Both the decrease in stem density, smaller leaves and lower NSC concentrations with earlier snowmelt indicate that *S. herbacea* does not benefit from early snowmelt (and thus a longer growing season) with increased biomass production. This corresponds to Wijk (1986), who also found increasing stem density in *S. herbacea* under later snowmelt conditions, and to Sedlacek *et al.* (2015), who found larger leaf sizes in *S. herbacea* growing in late snowbeds relative to early-exposure sites. In the few alpine accelerated snowmelt studies available, few dwarf shrub species demonstrated any enhancement in vegetative growth, with only low-lying shrubs evergreen *E. nigrum* and *L. procumbens*, two species characteristic to exposed microhabitats, showing increased stem elongation in

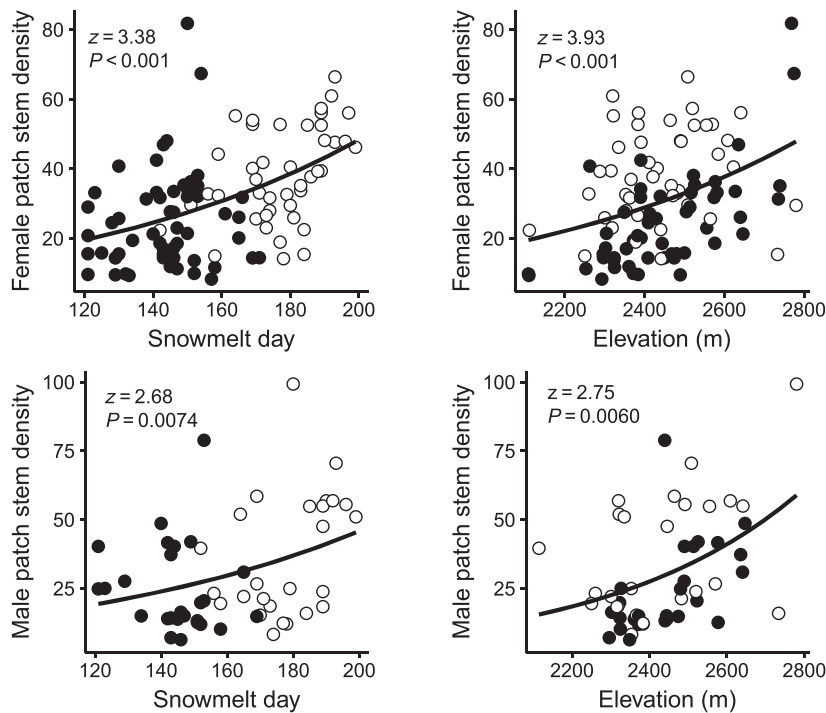


Fig. 4. Patch stem density for female and male *Salix herbacea* averaged for both plot and year along the elevation and snowmelt gradient over 3 years on three mountains in Davos, Switzerland. Black points represent data from ridge microhabitats, while white points represent snowbeds. Data are fitted with a Poisson regression curve when the relationship is statistically significant.

response to early snowmelt (Wipf, Stoeckli & Bebi 2009; Wipf 2010). It is unlikely competitive exclusion explained low stem density, since a concurrent neighbour removal study covering the same elevational transects demonstrated no evidence of competitive restriction of *S. herbacea* by the surrounding vegetative community on early-snowmelt microhabitats or at lower elevations (Wheeler *et al.* 2015). We suggest that competitive plant–plant interactions are not the factor reducing *S. herbacea* growth in earlier snowmelt sites, and that the lower stem density observed on early-exposure ridges may be mainly attributable to lower spring temperatures and frost events, which have been associated with reduced growth in many alpine shrubs. Wipf, Stoeckli & Bebi (2009) found a general decrease in shoot growth in *E. nigrum*, *Vaccinium myrtillus* and *V. uliginosum* under accelerated snowmelt which was often correlated with frost exposure. Similarly, Gerdol *et al.* (2013) found reduced leaf size and leaf number in *V. myrtillus* as a result of frost injury under early snowmelt. It is unlikely that overwinter temperatures significantly impacted shrub performance, due to relatively persistent snowcover on the majority of plots. Overwinter soil temperatures were thus buffered, and generally stable near 0 °C. Soil temperatures below –5 °C were uncommon (measured on 14 of 92 loggers overwinter), and the lowest overwinter temperature recorded was –19.6 °C. *Salix* species are generally extremely cold-tolerant when hardened for winter (Sakai & Larcher 1987), so it is unlikely that the *S. herbacea* patches sustained any significant cold damage overwinter. Indeed, stem density and flowering proportions for *S. herbacea* exposed to winter minimums below –5 °C were similar to the mean values for all microhabitats. This suggests that poorer growth in *S. herbacea* under early

snowmelt is a response to exposure to unfavourable abiotic conditions during the growing season, and that growth responses to earlier snowmelt in prostrate shrubs may be similar to taller dwarf shrubs.

LEAF DAMAGE MORE FREQUENT UNDER EARLIER SNOWMELT

Greater likelihood of leaf damage under earlier snowmelt conditions can impact plant fitness in the short term by reducing growth, flowering and fruit production (Gerdol *et al.* 2013; Viana *et al.* 2013; Wheeler *et al.* 2015) and in the long term, if repeatedly hit, as stored resources become depleted (Molau 1997). Further, biomass damage under earlier snowmelt may have a critical effect on community structure. Despite showing an increase in fungal phytopathogen infection with longer snowcover, Olofsson *et al.* (2011) still demonstrated that a fungal pathogen outbreak triggered by changes in snowmelt timing can alter the entire community structure in arctic shrub heathland. Our results showed an increasing likelihood of leaf damage by both insect herbivores and fungal pathogens with earlier snowmelt at the microhabitat scale. Similarly, Roy, Gusewell & Harte (2004) found increasing plant damage under earlier snowmelt for most herbivores and pathogens examined in an alpine meadow system, likely due to increasing periods available for herbivore growth and reproduction. Our results suggest that early-exposed shrubs are more likely to emerge concurrently with the primary leaf-damaging agents, while shrubs emerging later may avoid the peak populations of these herbivorous insects and fungal pathogens, and may thus be tolerant by avoidance of exposure. Further, *S. herbacea* show reduced flowering on later snowmelt sites in the year following leaf

damage by herbivores or fungal pathogens (Wheeler *et al.* 2015), indicating that vegetative damage may have an indirect but detrimental impact on fitness.

IMPLICATIONS FOR PROSTRATE SHRUBS UNDER CLIMATE CHANGE

The snow-shrub story represents an ongoing research topic in tundra biome responses under global change, and our results suggest the prostrate dwarf shrub *S. herbacea* demonstrates critical reductions in performance, particularly under earlier snowmelt. Overall, warmer mean temperatures at lower elevations conferred no real performance benefits, and earlier snowmelt, with associated frequent frost events, reduced performance in multiple key functional and fitness traits for both male and female plants. Our results highlight the importance of considering both climate warming and snowmelt timing as separate and sometimes interactive drivers provoking differential responses in phenological, reproductive and growth traits in low-lying dwarf shrubs, and that these drivers may outweigh other potential community structural mechanisms such as competition. Our results further suggest a detrimental impact of earlier spring snowmelt on performance and fitness in *S. herbacea*, and these results could potentially be generalized to other low-lying prostrate shrub species in arctic and alpine tundra biomes, as they too may be closely ecologically linked with snowmelt. Earlier spring snowmelt timing, as a result of warming climates, may thus represent one of the most important mechanisms leading to the widespread reductions in dwarf shrub communities observed by Elmendorf *et al.* (2012) in tundra ecosystems.

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Conflict of interest

The authors declare they have no conflict of interest.

Data accessibility

Data are deposited in the Dryad repository: <http://dx.doi.org/10.5061/dryad.6js40> (Wheeler *et al.* 2016).

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Supporting Information

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

Figure S1. Frequency of frost events during the growing season (snowmelt to first three consecutive days of new snowcover) in 2011 and 2012 along the elevation and snowmelt gradient on one mountain near Davos, Switzerland.

Figure S2. Proportion of stems fruiting for *Salix herbacea* with increasing frost frequency during the growing season in 2011 and 2012 on three mountains in Davos, Switzerland.

Figure S3. Leaf damage probability by fungi (left panel) and insect herbivory (right panel) for *Salix herbacea* during the 2011, 2012 and 2013 growing seasons along the elevation and snowmelt gradient on three mountains in Davos, Switzerland.