

**The snow and the willows: the ecological responses of  
the alpine dwarf shrub *Salix herbacea* to climate change**

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## **Chapter 1**

### **General Introduction**

#### *Climate change in alpine tundra*

Mountain ecosystems represent a terrestrial region characterized by high biodiversity, due to factors such as paleogeographical processes, spatial isolation driving migration and evolution, in addition to the availability of strongly heterogeneous microhabitat types, leading to a high degree of specialization (Körner 2003, Casazza et al. 2005, Scherrer and Körner 2010). Climate change, however, is expected to drive strong, pronounced environmental shifts in alpine ecosystems, leading to potential reductions in alpine biodiversity (e.g. Nogues-Bravo et al. 2006, Engler et al. 2011). Temperatures in the Swiss Alps have warmed on average by 1.5K between 1974 and 2004 (Rebetez and Reinhard 2008). These increasing average air temperatures lead to less precipitation falling as snow overwinter, with lower snow accumulation and accelerating snowmelt in spring leading to a longer growing season (Beniston et al. 2003). Declines in snowpack and earlier snowmelt as a result of warming have already been observed and modelled in many mountain ecosystems (e.g. Pederson et al. 2011, Harpold et al. 2012), including the Swiss Alps (Rixen et al. 2012, Alaoui et al. 2014).

As a response to climate change, Thuiller et al. (2005) predicted biodiversity loss of up to 60% in European mountain ecosystems before the end of the century, though models operating at broad spatial scales may over-predict plant extinction, as mountain ecosystems have highly heterogeneous microtopography where environmental predictors may vary on a fine spatial scale (Randin et al. 2009). Despite disagreements on the scale of species loss, however, many alpine ecosystems and associated plant communities are already showing effects of climate change. Multiple studies (e.g. Pauli et al. 2012, Wipf et al. 2013) have documented increasing

biodiversity on mountain summits in Europe, since plants migrate to higher altitudes as warming temperatures reduce low-temperature filters. Further, species upper distribution limits, and thus new source populations, are also migrating upward (e.g. Walther et al. 2005, Lenoir et al. 2008, Frei et al. 2010). This novel species migration has in turn been sometimes been associated with declines in high-alpine species (Pauli et al. 2007, Lesica 2014). Finally, Dullinger et al. (2012) have predicted extinction debts for many alpine species, driven by the lag between loss of climatically suitable habitat and species disappearance in alpine habitats. Thus, since alpine species diversity and biomass are generally driven by the interactions of climatic conditions and species interactions (Kikvidze et al. 2005), changing climatic conditions and shifting species interactions may lead to complex changes in alpine plant communities. Thus, it is critical to understand the interactions between ongoing changes in temperature, snow cover, community interactions and species-specific responses to climate change in order to predict the effects of changing climate regimes on alpine plants.

### *Snowmelt, warming and alpine communities*

In alpine habitat, highly heterogenous microtopography leads to snowmelt patterns demonstrating strong spatial consistency from year to year (Körner 2003) and classical ecological studies have extensively examined the role in snow cover as the fundamental control over the distribution and structure of alpine plant communities (summarized in Körner 2003). Alpine communities are generally structured based on species tolerance for snow pack duration, and thus growing season length, with snow also having an indirect effect on soil quality and neighbor interactions (Galen and Stanton 1995). In addition, many alpine plant species are closely phenologically linked with snowmelt timing (Keller and Körner 2003, Wipf and Rixen 2010), as

snowmelt generally marks the beginning of the growing season for alpine species (Schöb et al. 2009). Advances in snowmelt timing can thus represent a critical abiotic change for alpine plant communities, as it increases the duration of the photosynthetic period (Inouye 2008), and can lead to increased drought exposure, spring frost events and damage by herbivores and phytopathogens (Roy et al. 2004, Giménez-Benavides et al. 2007, Inouye 2008). Warming can also strongly impact alpine plant communities; it may increase productivity, but can also lead to the loss of certain thermal habitats optimal for alpine species (Scherrer and Körner 2010), in addition to altering the biotic interactions that play an important role in structuring alpine plant communities (HilleRisLambers et al. 2013).

#### *Alpine shrub responses to a changing climate*

Dwarf shrubs in temperate mountains represent an interesting case of alpine vegetation: they are woody, low-lying, often clonal, and can be very long-lived; Körner (2003) described some clonal dwarf shrubs as “functionally immortal.” Alpine shrubs are common and often a dominant species in alpine plant communities. Understanding how long-lived shrub species react, in addition to their potential to respond to changing climates, will help us project the future structure and functioning of alpine plant communities.

In both alpine and Arctic ecosystems, shrub expansion has been extensively documented as a result of changing climatic conditions (e.g. Myers-Smith et al. 2011, Hudson et al. 2011, Elmendorf et al. 2012). Shrub expansion has multiple impacts on ecosystem functioning in the tundra, including snow trapping, warming through albedo alteration, and alterations of soil temperature and moisture (Myers-Smith et al. 2011) and thus is a topic of critical ecological importance. However, dwarf shrubs have shown widespread reductions under climate changes

that have led to expansion of taller shrubs in the Arctic (Elmendorf et al. 2012). Thus, understanding the mechanisms of dwarf shrub responses to the warming and accelerated snowmelt characteristic to alpine climate change remains an important research question.

To predict how alpine shrub communities will respond to climate change, we must understand the complex interplay between multiple biotic and abiotic drivers of shrub response. *Salix herbacea*, the snowbed willow, is a prostrate dwarf shrub occupying a wide range of microhabitat types in in arctic, subarctic and alpine ecosystems (Beerling 1998). In the Alps of eastern Switzerland, it covers a long elevational gradient (~800 m) and a wide range of snowmelt microhabitats with distinct plant communities, from early-exposure ridges with little winter snow cover to late-lying snowbeds with very short growing seasons. Thus, it represents an excellent candidate species for examining fitness and performance of alpine dwarf shrubs in response to microclimatic conditions, and quantifying how a common dwarf shrub will respond to accelerating spring snowmelt timing, warmer growing seasons, including associated shifts in plant-plant interactions within the alpine plant community. Further, we can examine the frequency and impacts of damaging phenomena like spring frosts, herbivory, and phytopathogen infection, and predict how these events may influence shrub performance changing climate conditions.

### *This doctoral thesis*

The main objective of this doctoral thesis was to determine how the common dwarf shrub *Salix herbacea* will respond to climate change. Specifically, we posed the following questions:

1. Which functional traits of *S. herbacea* are primarily controlled by the temperatures during the growing season, and which are mainly affected by snowmelt timing?
2. Which traits demonstrate plastic responses to changes in snowmelt timing, and does *S. herbacea* demonstrate any evidence of a home-site advantage which could suggest local adaptation to particular microhabitat conditions?
3. How are trait responses of *S. herbacea* to warming and snowmelt acceleration mediated by neighbor interactions with the alpine plant community?
4. Does spring frost damage represent a significant threat to *S. herbacea* and the alpine shrub community under climate change?

#### *Study sites and experimental design*

Our field study sites were established in 2011 on three mountains near Davos, in the eastern Swiss Alps: Jakobshorn (46°46' N, 09°50' E, 2100 to 2600 m asl), Schwarzhorn (46°44' N; 09°57' E, 2380 to 2780 m asl) and Wannengrat (46°48' N, 09°46' E, 2280 to 2640 m asl). At 2090 m asl on Jakobshorn, at the Stillberg climate station, average annual air temperature and precipitation from 1975-2012 have been recorded as 2.06 °C and 1150 mm, respectively, with 48% of yearly precipitation falling between June and September (Rixen et al. 2012). Snowfall may occur in all months, but standing snow cover usually occurs from mid-October until late in May (Wipf et al. 2009). During almost four decades of observation, mean air temperatures during the summer months (June, July and August) at Stillberg have increased at a rate of 0.58°C per decade, with a corresponding spring snowmelt acceleration of 3.5 days per decade (Rixen et al. 2012).



In a space for time substitution study, we established three elevational transects, one on each mountain, where at ten elevational bands, we placed study plots (c. 3x3 m) in two early-exposure ridge microhabitats and two late-exposure snowbed microhabitats. All microhabitat sites had similar primarily NE exposure and covered the main elevational range of *S. herbacea*. In each of these 120 study plots, we selected four *S. herbacea* patches of 10 cm diameter, for a total of 480 patches, and marked them for long-term monitoring. For all study patches, we monitored shrubs weekly from first exposure from snow until leaf senescence during spring and summer in 2011, 2012 and 2013. Every week, we visited patches to record leaf developmental status, proportion of stems flowering per patch, proportion of stems fruiting per patch and presence of leaf tissue damage by herbivores and fungi. Once per season, we recorded stem number and mean leaf area for each patch. We performed non-structural carbohydrate (NSC) analysis on wood tissue (stems) in 2012 to determine seasonal carbon accumulation potential. The results of this study are detailed in Chapter 2.

We then established a neighbor removal experiment in 60 of the abovementioned study plots (20 per mountain, 10 ridges and 10 snowbeds). Sixty patches of *S. herbacea* were selected, and all neighbouring vegetation in the surrounding 40 cm was removed in 2011, in order to determine the effects of neighbor interactions along snowmelt and elevation gradients on *S. herbacea* in 2013. We discuss this study in Chapter 4. Further, we conducted a freezing resistance study within one transect in this study. On eight plots on the Jakobshorn transect in 2012, we sampled leaf tissue from four alpine shrubs species for a laboratory study to determine freezing resistance and relative vulnerability to spring frost events based on elevational position, microhabitat of origin and species. This study is discussed in Chapter 6.

At the base of the Schwarzhorn transect, we established a reciprocal transplant experiment to examine trait plasticity and determine local adaptation in *S. herbacea* to home microhabitat conditions. On twelve paired ridge-snowbed sites, we reciprocally transplanted 336 *S. herbacea* turfs in 2011 and monitored phenology, sexual reproduction, stem number, leaf size and leaf damage over the following two years. The details of this study are examined in Chapter 3.

### *Specific objectives of each chapter*

#### *Chapter 2: The snow and the willows: Accelerated spring snowmelt reduces performance in the alpine shrub *Salix herbacea**

In this chapter, we examined the relative importance of warming (using temperature lapse along elevation gradients) and growing season length (using natural snowmelt range between microhabitats) as drivers of trait variation in *S. herbacea* over three consecutive years. Current literature suggests that changes in shrub abundance in alpine and arctic tundra ecosystems are primarily driven by these environmental factors, but as warming and earlier snowmelt can be decoupled, we need to understand the effects of both factors on tundra plants to predict future vegetation change. This study used a space for time substitution along elevation and snowmelt gradients to determine the effect of warming and accelerated snowmelt on phenology, sexual reproduction, growth, carbon balance and damage probability. Specifically, we hypothesized that earlier snowmelt would accelerate phenological timing, but fail to increase vegetative growth or sexual reproduction, while low-elevation warming would benefit sexual reproduction and vegetative production.

*Chapter 3: The response of the alpine dwarf shrub Salix herbacea to altered snowmelt timing: lessons from a multi-site transplant experiment*

This chapter represents a co-first authorship with Janosch Sedlacek (PhD candidate, University of Konstanz, Germany). In this study, we examined phenotypic plasticity in response to snowmelt change and home-site advantage in *Salix herbacea*. We tested how *S. herbacea* responded to shifts in snowmelt timing by reciprocally transplanting shrub turfs between early-exposure ridge and late-exposure snowbed microhabitats, then monitoring phenological, morphological and fitness traits as well as leaf damage during the following seasons. This is an ecologically important question, as it is critical to understand whether phenotypic plasticity can help to adapt to changing snowmelt conditions, and whether a home-site advantage to local microhabitat conditions might initially slow shrub response.

*Chapter 4: With a little help from my friends: community facilitation for dwarf shrub Salix herbacea under climate change*

In this chapter, we examined the influence of community interactions relative to abiotic environmental conditions on phenological, functional, fitness traits and damage probability in *Salix herbacea*. We specifically hypothesized that plant-plant interactions would influence traits that have the potential for rapid response to changes in neighbor density, such as leaf size. Further, we hypothesized that neighbours reduce by sheltering the more prostrate *S. herbacea*. Last, we hypothesized that in accordance with the stress gradient hypothesis, biotic interactions would shift from competitive to facilitative as elevation increases and snow melts earlier, as environmental stress increases.

*Chapter 5: Increased spring freezing vulnerability for alpine shrubs under early snowmelt*

In the final chapter, we examined the role spring frost events play in structuring alpine shrub communities. We sampled biomass from four different shrub species at early and late snow exposure sites along the Jakobshorn elevational transect to investigate the effect of snowmelt time and elevation on shrub growth and risk of exposure to lethal spring and early summer frost in four alpine dwarf shrubs. We tested freezing resistance of live mature leaf tissue in the laboratory to determine whether shrubs growing in early snowmelt and high-elevation sites were more frost-resistant than the same species growing at low elevation and late snowbed sites. We hypothesized that despite leading to a longer growing season, earlier snowmelt would not enhance shrub growth because earlier snowmelt would increase potential exposure to damaging spring frosts.

## Chapter 2

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

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Phenological stages of *S. herbacea*, photos by Julia Wheeler

## Summary

Current changes in shrub abundance in alpine and arctic tundra ecosystems are primarily driven by warming, changes in snow cover and growing season length. However, while taller shrub communities are expanding, dwarf shrubs show reductions under climate warming, and the mechanisms driving dwarf shrub reductions (such as warming temperatures or accelerated spring snowmelt) may be highly complex. To determine and disentangle the response of a widespread prostrate shrub to both climate warming and changes in snowmelt time, we investigated a wide range of phenological, growth and fitness traits in 480 patches of *Salix herbacea*, a common alpine and arctic prostrate species, along its elevational and snowmelt microhabitat range over three years in a space-for-time substitution.

Earlier snowmelt was associated with longer phenological development periods, an increased likelihood of herbivory and fungal damage, fewer stems and no increase in end-of-season wood reserve carbohydrates. Furthermore, while early snowmelt was associated with an increased proportion of flowering stems, the number of fruiting stems was not, as fruit set decreased significantly with earlier snowmelt. Warmer temperatures at lower elevations were associated with decreases in stem number and increases in proportion of stems fruiting.

## Synthesis

Our study indicates that most of the measured traits of *S. herbacea* are influenced by snowmelt, and that earlier spring snowmelt mainly reduced performance in *S. herbacea*. The most likely mechanisms for many of the observed patterns are related to adverse conditions in the early growing season. Reductions in clonal 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 warming temperatures for *S. herbacea*, particularly as warming was associated with lower clonal growth. Under typical global change scenarios for arctic and alpine tundra, where growing seasons are both warmer and longer as a result of accelerated snowmelt, we conclude that early snowmelt represents an important mechanism reducing fitness and performance in a common and widespread dwarf shrub.

## 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). However, shrub encroachment is mainly driven by expansion of taller shrubs (Elmendorf et al. 2012); in contrast, dwarf shrubs (<15cm height) show widespread reductions as a response to warming (Elmendorf et al. 2012), with simulations showing up to 60% habitat loss for prostrate dwarf shrub tundra (Kaplan and 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 (less than 15 cm, and often prostrate growth forms), many dwarf shrubs are strongly ecologically and phenologically dependent on snowmelt timing (Wipf et al. 2009, Wipf and 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 warming and altered snowmelt on taller deciduous dwarf shrubs (e.g. Anadon-Rosell et al. 2014, Gerdol et al. 2013, Rixen et al. 2012, Wipf 2010), the responses of prostrate shrubs, particularly deciduous species, to warming and snowmelt change have been relatively understudied (but see Clemmensen and Michelsen 2006, Kudo et al. 2010).



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 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 augmented flower production under warming, but no increase in seedling establishment (Suzuki and 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*, *V. myrtillus*; Wipf et al. 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 helpful in understanding growth limitation in trees at treeline (e.g. Körner 2003a, Hoch and Körner 2012) but have rarely if ever been examined in low alpine shrubs. Studies examining shrub responses to global change typically focus on a narrow range of traits (e.g. relative growth rate, flowering, but see Wipf et al. 2009 and Wipf 2010, which examined survivorship, phenology, growth and flowering), which may represent an unsuitable approach to examining responses in typically long-lived and clonal tundra shrubs. Studies also typically examine shrub responses to either altered snowmelt time or warming, but not both global changes concurrently.

Snowmelt timing and warming may also alter the multitrophic biotic interactions for the whole shrub community, particularly as it affects exposure to herbivores and pathogens (Roy et al. 2004). Again, predicting dwarf shrub responses is difficult, as later-melting snow cover may

shelter low-lying shrubs by acting as a barrier to these damage agents or serve to intensify later herbivory damage to shrub leaf tissue (Roy et al. 2004). Timing of snowmelt will also control the temperature regime, as accelerated 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 widespread and often dominant prostrate dwarf shrub common in arctic and alpine tundra, and represents one species facing both warming climates and earlier snowmelt. *S. herbacea* is an optimal 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 between sub-populations 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 sub-populations along snowmelt or elevational gradients, although genetic differentiation at loci under strong local selection cannot be ruled out entirely (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 and 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 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: in males and females, we examined phenophase timing and developmental period, vegetative growth, C balance (accumulation of non-structural carbohydrates during the growing season) and the likelihood of leaf damage by insect herbivores and fungal phytopathogens. Further, for females, we investigated sexual reproductive capacity.

The objectives of this study were to determine how each set of traits responded to a) earlier snowmelt timing and thus an elongated growing season and b) warmer seasonal 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, 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 phenological, reproductive, growth and carbon balance traits, in addition to probability of herbivory and tissue damage by phytopathogen elements. The results of this study will thus help disentangle 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.

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 to 2600 m asl), Schwarzhorn (46°44' N; 09°57' E, 2380 to 2780 m asl) and Wannengrat (46°48' N, 09°46' E, 2280 to 2640 m asl) all had similar primarily NE exposure and covered the main elevational range of *S. herbacea*. At 2100 m asl 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-years measurement period, air temperatures during the warmest months (June-August) have increased by 0.58°C per decade, and spring snowmelt has accelerated 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 (c. 3x3 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. 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 et al. 2007).

We monitored shrubs weekly from snowmelt until leaf senescence during the 2011, 2012 and 2013 growing seasons. We recorded snowmelt timing, leaf developmental status (bud break,

rolled, open), proportion of stems flowering per patch and proportion of stems fruiting (newly mature fruit) per patch 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 belowground 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. To support this argument, a greenhouse pot experiment showed above-ground stem production correlated strongly with belowground biomass production ( $r=0.86$ ,  $p<0.0001$ ; Sedlacek unpublished data).

We performed non-structural carbohydrate (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 hours within a maximum of six hours 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 et al. (2002). NSC were 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 % dry wood mass.

We recorded soil temperature (5 cm below the surface, 2 hour 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). We calculated growing degree days (GDD) above 5°C as derived from the two-hour 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.

### *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 centered 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 strongly correlated. Thus, we selected elevation to act as a general proxy for growing season temperature effects. The nested random factors were study plot, elevational band (an alphanumeric category indicating the proximity of four study plots per elevational band) and transect, with year as an additional crossed random effect.

Variation in all traits was analysed using generalised linear mixed models. For phenological development time (first day of leaf expansion, flowering and fruiting, and the period of development from snowmelt to each phenophase), proportion of stems flowering per patch, proportion of stems fruiting per patch, wood NSC, wood starch, wood sugar, leaf area and stem density, we used a normal error distribution. For fruit set, and the presence/absence of leaf damage (from herbivory and fungi), we used a binomial error distribution. Fixed terms were

snowmelt day, elevation (both centered to a mean of zero; Schielzeth 2010), and sex, for traits not related to reproduction. The two-way interactions of all fixed terms were included. The nested random factors were plant patch, study plot, elevational band and transect with year as an additional crossed random effect. Response variables were transformed to ensure normality of residuals when necessary, and standard diagnostic plots were used when appropriate to confirm assumptions of linearity and homoscedasticity. Models examining flowering, fruiting, and fruit set only included data from female plants, as the number of flowering male plants was much lower. All statistical analyses were carried out in R v.2.15.1, using package lme4 (Bates et al. 2013), with p-values generated using MCMC resampling.

## 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. Mean soil temperatures during both the full growing season (i.e. from date of snowmelt to the end of August) and in the first 30 days following snowmelt increased significantly with later snowmelt ( $t=6.97$ ,  $p<0.0001$ ;  $t= 3.554$ ,  $p=0.00065$ ; Figure 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 ( $t=-3.715$ ,  $p=0.00082$ ;  $t=-1.638$ ,  $p=0.11$ ; Figure 1). There was a significant interaction effect between snowmelt time and elevation on mean soil temperature in the first 30 days after snowmelt ( $t=-3.050$ ,  $p= 0.0034$ ) and a marginally significant interaction effect of snowmelt time and elevation on mean soil temperature for the whole growing season ( $t=-1.921$ ,  $p=0.058$ ).

### *Phenology and developmental timing*

The first day of occurrence for each monitored phenological stage was controlled by snowmelt day, with significantly later first occurrence of each phenophase with later snowmelt (Table 1 and Figure 2). Elevation only affected phenology through an interaction with snowmelt timing. For first day of leaf expansion and flowering, there was a significant snowmelt day-by-elevation interaction, with later leaf expansion and flowering day occurring at higher elevation sites with later snowmelt (Table 1). The developmental period to each phenophase (number of days from snowmelt day to first day of leaf expansion, flowering and fruiting) was significantly shorter with later snowmelt (Table 1 and Figure 2), but was not affected by elevation (Table 1). First day of leaf expansion and development time to leaf expansion were not significantly different between sexes (Table 1), and there were no significant sex-driven differences in timing of leaf development along the elevation or snow gradient (Table 1).

Temperatures accumulated at the days of leaf opening and flowering (i.e., GDD from snowmelt to leaf open, GDD from snowmelt to flowering) were not significantly different along elevation or snowmelt gradients (leaf open GDD along elevation gradient:  $t=-1.41$ ,  $p=0.16$ ; along snowmelt gradient:  $t=-1.48$ ,  $p=0.14$ ; 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. Temperature accumulated at fruiting phenophase (day of fruiting GDD) significantly decreased with later snowmelt but not with elevation ( $t=-2.22$ ,  $p=0.029$ ;  $t=0.22$ ,  $p=0.82$ ).

### *Reproduction*



The proportion of stems flowering decreased significantly with later snowmelt (Table 1 and Figure 3), and tended to decrease with elevation, although this was not significant (Table 1 and Figure 3). The proportion of stems fruiting decreased significantly with elevation (Table 1 and Figure 3) and was not affected by snowmelt time (Table 1 and Figure 2). The difference between flower and fruit production along the snowmelt gradient was explained by fruit set, which increased significantly with later snowmelt (Table 1). Hence, the resource allocation to more flowers 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).

#### *Vegetative growth*

The mean area per leaf was primarily controlled by sex, with females having larger leaves (female mean leaf size =82.11 mm<sup>2</sup>, male mean leaf size =76.16 mm<sup>2</sup>,  $t=2.45$ ,  $p=0.015$ ). Leaf area was not affected by snowmelt timing or elevation (Table 1). Shrub stem number per area did not differ significantly between the sexes, and increased with both elevation and later snowmelt (Table 1 and Figure 4;  $N=919$ ,  $t=4.37$ ,  $p<0.0001$ ;  $N=919$ ,  $t=2.72$ ,  $p=0.0066$ ). In addition, there was a significant interaction between sex and elevation, with male stem density increasing more rapidly with elevation (Table 1).

#### *Non-structural carbohydrate concentrations*

Late-season wood tissue NSC and starch concentrations in stem wood were relatively high overall (NSC mean=21.65 % dry mass, SE=0.25; starch mean=14.73 % dry mass, SE=0.23), demonstrating that carbon was likely not limited across the entire gradient. Neither NSC nor its component starch changed significantly with elevation or later snowmelt (Table 1), while

component low-molecular-weight sugars increased with elevation (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 (Table 1). 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 external factors in *S. herbecea* were strongly associated with snowmelt timing, and to a much lesser extent, with elevation. Phenological development began earlier but lasted longer with earlier snowmelt, with a longer period required to develop to the leaf expansion, flowering and fruiting phenological stages. Shrubs growing on earlier exposure sites produced proportionately more flowers than later-exposed shrubs, but demonstrated reduced fruit set, so that there was no difference in fruit production along the snowmelt gradient. Further, fruit production decreased with increasing elevation. Clonal reproduction, as measured by stem number, was reduced under earlier snowmelt and at lower elevations. Non-structural carbon concentrations in end-of-season wood tissue were high and similar along snowmelt and elevation gradients. Leaf damage by insect herbivores and fungal phytopathogens was more likely to occur

with earlier snowmelt. Our results suggest that accelerated spring snowmelt time, as opposed to mean growing season temperatures, represents the most important driver of trait variation in *S. herbacea*. The poorer performance of growth and reproductive traits under earlier snowmelt, in addition to increasing damage probability, suggests a potential reduction of fitness in *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 and Suzuki 1999, Molau et al. 2005, Huelber et al. 2006). This is generally supported by our data, where growing-degree days (GDD) accumulated at the first day of leaf open and 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. Photoperiodism as a driver here is unlikely, as daylength 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 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 et al. 2009). In our study sites, exposure to freezing is generally spatially and temporally restricted to earlier snowmelt sites, and is likely absent on later-melting snowbeds (Wheeler et al. 2014). 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).

#### *Reduced fruit production and vegetative growth under early snowmelt*

Localized snowmelt time and, correspondingly, growing-season length 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 fruit success. Montesinos et al. (2012) found also a 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. 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 exposed to spring temperatures cold enough to kill even leaves on early exposure sites (Wheeler et al. 2014). 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*.

Correspondingly, the increase in fruiting with decreasing elevation suggests a positive response to warmer temperatures on the larger scale. In both dwarf and taller shrubs

*Arctostaphylos alpina* and *Vaccinium uliginosum*, enhanced warming has been shown to increase reproductive output (Suzuki and Kudo 2005). This suggests that warming temperatures might increase fruit production on earlier-exposure sites in the future, meaning that the current allocation for increased flowering on ridges could pay off, particularly on lower, warmer early-exposure sites. However, this scenario might be counter-balanced, as flower-damaging spring freezing events are expected to become more common under earlier snowmelt (Inouye 2008, Wipf et al. 2009, Gerdol et al. 2013). Further, field germination rates for *S. herbacea* are likely extremely low (<0.02% on ridges, <2% on snowbeds; Wheeler, unpublished data), suggesting clonal growth may represent a more important reproductive pathway.

Both the decrease in stem density and unchanged 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. In the few alpine accelerated snowmelt studies available, few dwarf shrub species demonstrated any enhancement in vegetative growth, with only low-lying shrubs evergreen *Empetrum nigrum* and *Loiseleuria procumbens*, two species characteristic to exposed microhabitats, showing increased stem elongation in response to early snowmelt (Wipf et al. 2009, Wipf 2010). Low stem density could be explained by competitive exclusion by shrubs species on earlier snowmelt sites and at warmer lower-elevation sites, since the standing vegetation community at these microhabitats was shrub-dominated, and taller than *S. herbacea* (Nussbaumer 2012). However, 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. unpublished). That study showed the presence of neighbours did not reduce vegetative growth or fecundity in *S. herbacea*, and even

suggested the presence of neighbours facilitated *S. herbacea*, primarily by reducing herbivory damage. 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 et al. (2009) found a general decrease in shoot growth in *Empetrum 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. This suggests that poorer growth in *S. herbacea* under early snowmelt is a response to exposure to unfavourable abiotic conditions, 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) 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; Olofsson et al. (2011) demonstrated that fungal pathogen outbreaks 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 et al. (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.

### *Implications for prostrate shrubs under climate change*

The snow-shrub story represents an ongoing research question 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 temperatures at lower elevations conferred few performance benefits, and earlier snowmelt reduced performance in multiple key functional and fitness traits. 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. Our results further suggest a detrimental impact of accelerated 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. Acceleration in spring snowmelt timing, as a result of warming climates, may thus represent one of the most important mechanisms leading to reductions in dwarf shrub communities observed in tundra ecosystems.

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Table 1: Responses of *Salix herbacea* functional and traits from general linear mixed models to elevation, snowmelt time, sex and the two-way interaction effects of all factors over three years on three mountains in Davos, Switzerland. Response trait transformation is listed as square-root (sqrt), log or NA (for untransformed data), with estimate (est), t- or z-values and p-values (bolded when significant) from the mixed models.

Response trait	Transformation	Elevation	Snowmelt	Sex	Elevation*snow	Sex*elevation	Sex*snow
Day of leaf expansion	Sqrt	est=0.012 t=0.39 p=0.69	est=0.54 t=20.04 <b>p&lt;0.0001</b>	est=0.015 t=0.50 p=0.61	est=-0.026 t=-2.06 <b>p=0.05</b>	est=0.017 t=0.59 p=0.55	est=-0.016 t=-0.62 p=0.54
Day of flowering	Sqrt	est=0.016 t=0.68 p=0.50	est=0.55 t=26.44 <b>p&lt;0.0001</b>	NA	est=-0.035 t=-2.21 <b>p=0.028</b>	NA	NA
Day of fruiting	Sqrt	est=0.021 t=0.91 p=0.36	est=0.50 t=20.75 <b>p&lt;0.0001</b>	NA	est=-0.0031 t=-0.17 p=0.87	NA	NA
Time from snowmelt to leaf expansion	Sqrt	est=0.054 t=0.65 p=0.52	est=-1.11 t=-15.35 <b>p&lt;0.0001</b>	est=0.042 t=0.53 p=0.59	est=-0.056 t=-1.57 p=0.12	est=0.015 t=0.20 p=0.84	est=-0.042 t=0.59 p=0.56
Time from snowmelt to flowering	Sqrt	est=0.019 t=-0.34 p=0.73	est=-1.06 t=-20.47 <b>p&lt;0.0001</b>	NA	est=-0.064 t=-1.54 p=0.12	NA	NA
Time from snowmelt to fruiting	Sqrt	est=0.061 t=1.25 p=0.21	est=-0.98 t=-18.79 <b>p&lt;0.0001</b>	NA	est=-0.0012 t=-0.03 p=0.98	NA	NA
Proportion of patch flowering	Sqrt	est=-0.021 t=-1.75 p=0.081	est=-0.024 t=-2.58 <b>p=0.01</b>	NA	est=0.0037 t=0.44 p=0.66	NA	NA
Proportion of patch fruiting	Log	est=-0.12 t=-2.05	est=0.095 t=1.75	NA	est=0.074 t=1.70	NA	NA

Percent fruit set	NA	<b>p=0.041</b> est=-0.12 z=-1.14	p=0.081 est=0.39 z=3.63	NA	p=0.089 est=-0.073 z=-0.68	NA	NA
Mean leaf size	Log	p=0.26 est=-0.074 t=-1.68	<b>p=0.0003</b> est=0.055 t=1.41	est=0.11 t=2.45	p=0.50 est=-0.019 t=-1.18	est=-0.070 t=-1.59	est=0.0099 t=0.29
Stem density	Sqrt	p=0.094 est=0.84 t=4.37	p=0.16 est=0.29 t=2.72	<b>p=0.015</b> est=0.15 t=0.80	p=0.24 est=0.043 t=0.87	p=0.11 est=-0.41 t=-2.10	p=0.77 est=-0.10 t=-0.89
Wood NSC	NA	<b>p&lt;0.0001</b> est=0.25 t=0.5	<b>p=0.0066</b> est=0.94 t=1.48	p=0.42 est=-0.086 t=-0.14	p=0.38 est=-0.069 t=-0.26	<b>p=0.037</b> est=0.18 t=0.32	p=0.37 est=-0.11 t=-0.16
Wood starch	NA	p=0.62 est=-0.50 t=-0.99	p=0.14 est=0.74 t=1.18	p=0.89 est=-0.16 t=-0.28	p=0.79 est=0.080 t=0.31	p=0.75 est=0.45 t=0.83	p=0.88 est=-0.34 t=-0.49
Wood low MW sugar	NA	p=0.32 est=0.81 t=3.19	p=0.24 est=0.00023 t=0.01	p=0.78 est=0.22 t=0.84	p=0.76 est=-0.12 t=-0.94	p=0.41 est=-0.31 t=-1.25	p=0.62 est=0.37 t=1.21
Probability of leaf herbivory	NA	<b>p=0.0017</b> est=-0.13 z=-0.51	p=0.99 est=-0.55 z=-2.19	p=0.40 est=-0.26 z=-0.95	p=0.35 est=0.21 z=1.63	p=0.21 est=0.31 z=1.11	p=0.23 est=0.14 z=0.54
Probability of leaf fungi	NA	p=0.61 est=-0.089 z=-0.41	<b>p=0.028</b> est=-0.49 z=-2.54	p=0.34 est=0.064 z=0.31	p=0.10 est=-0.038 z=-0.41	p=0.26 est=-0.060 z=-0.27	p=0.59 est=0.29 z=1.40
		p=0.68	<b>p=0.011</b>	p=0.76	p=0.68	p=0.78	p=0.16

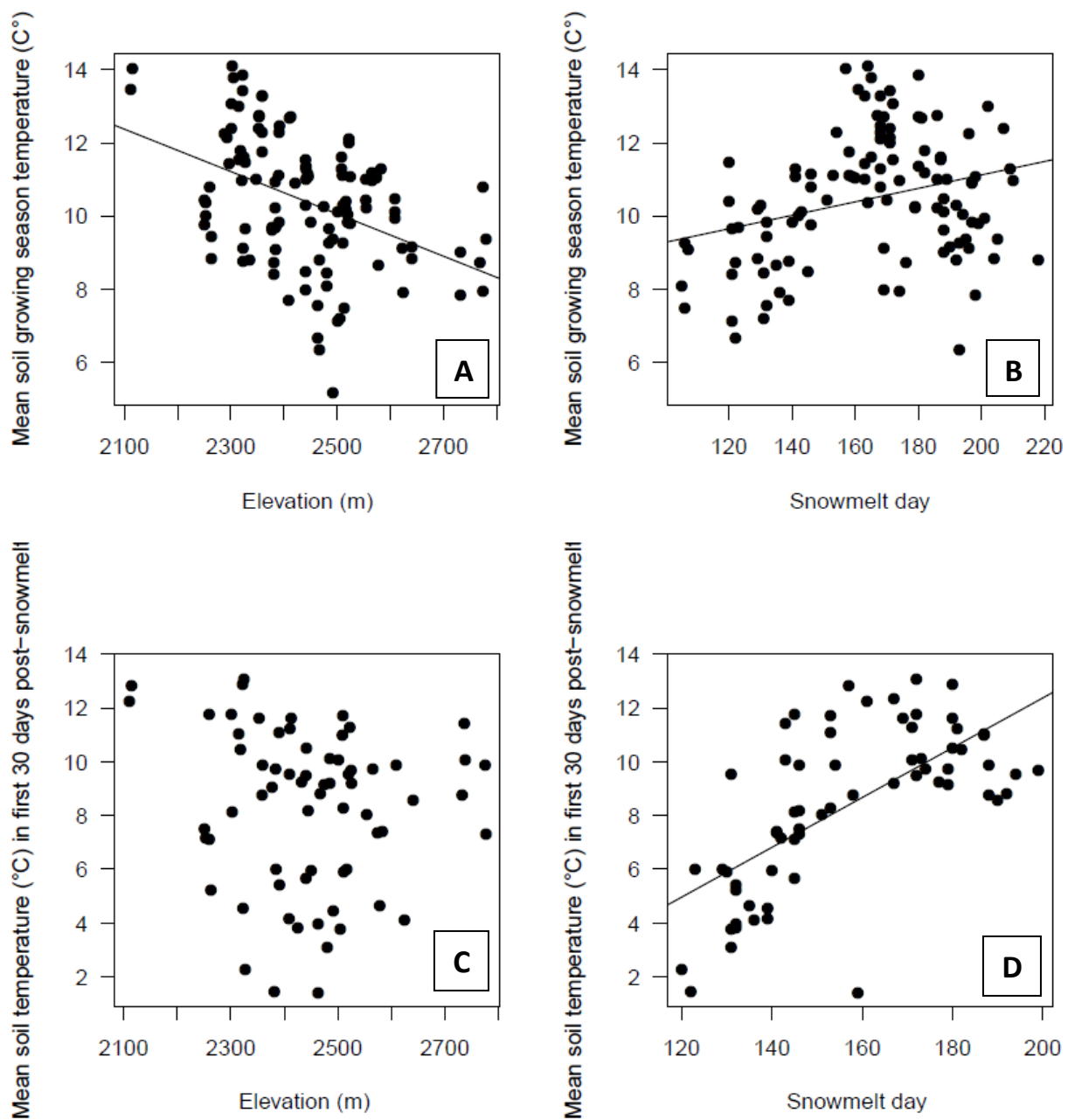


Figure 1: Mean soil temperature at a depth of 5 cm during the growing season (day of snowmelt until the end of August) along the A) elevation gradient and B) snowmelt gradient and mean soil temperature during the first 30 days after snowmelt along the C) elevation gradient and D) snowmelt gradient over two years on three mountains in Davos, Switzerland. Data are fitted with a curve when the relationship is statistically significant

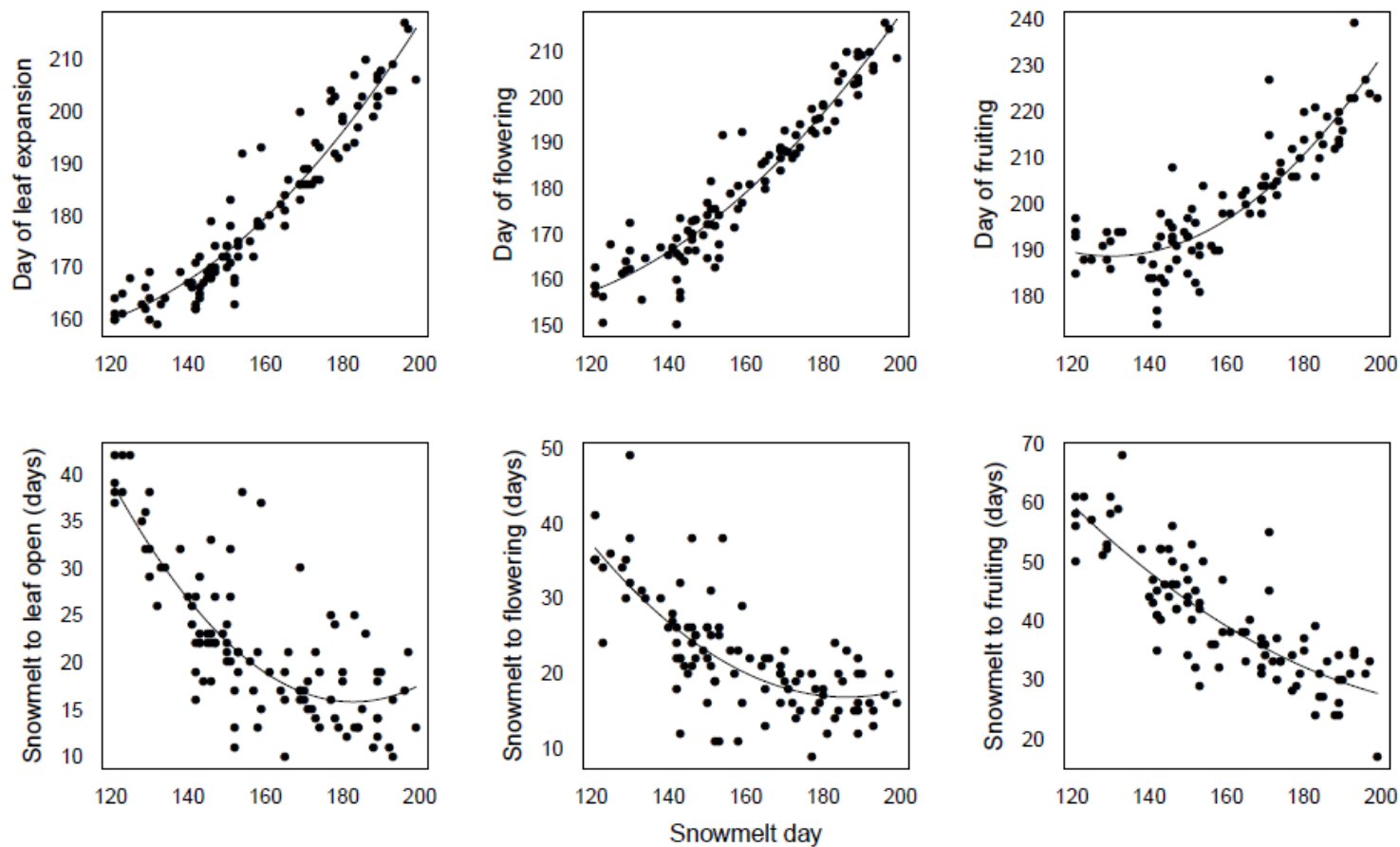


Figure 2: First day of leaf open, flowering and fruiting phenophase occurrence, and time required to develop to each phenophases for *Salix herbacea* averaged for both plot and year along the snowmelt gradient over three years on three mountains in Davos, Switzerland. Data are fitted with a curve extracted from model estimates when the relationship is statistically significant. Note different y-axis values for each panel.

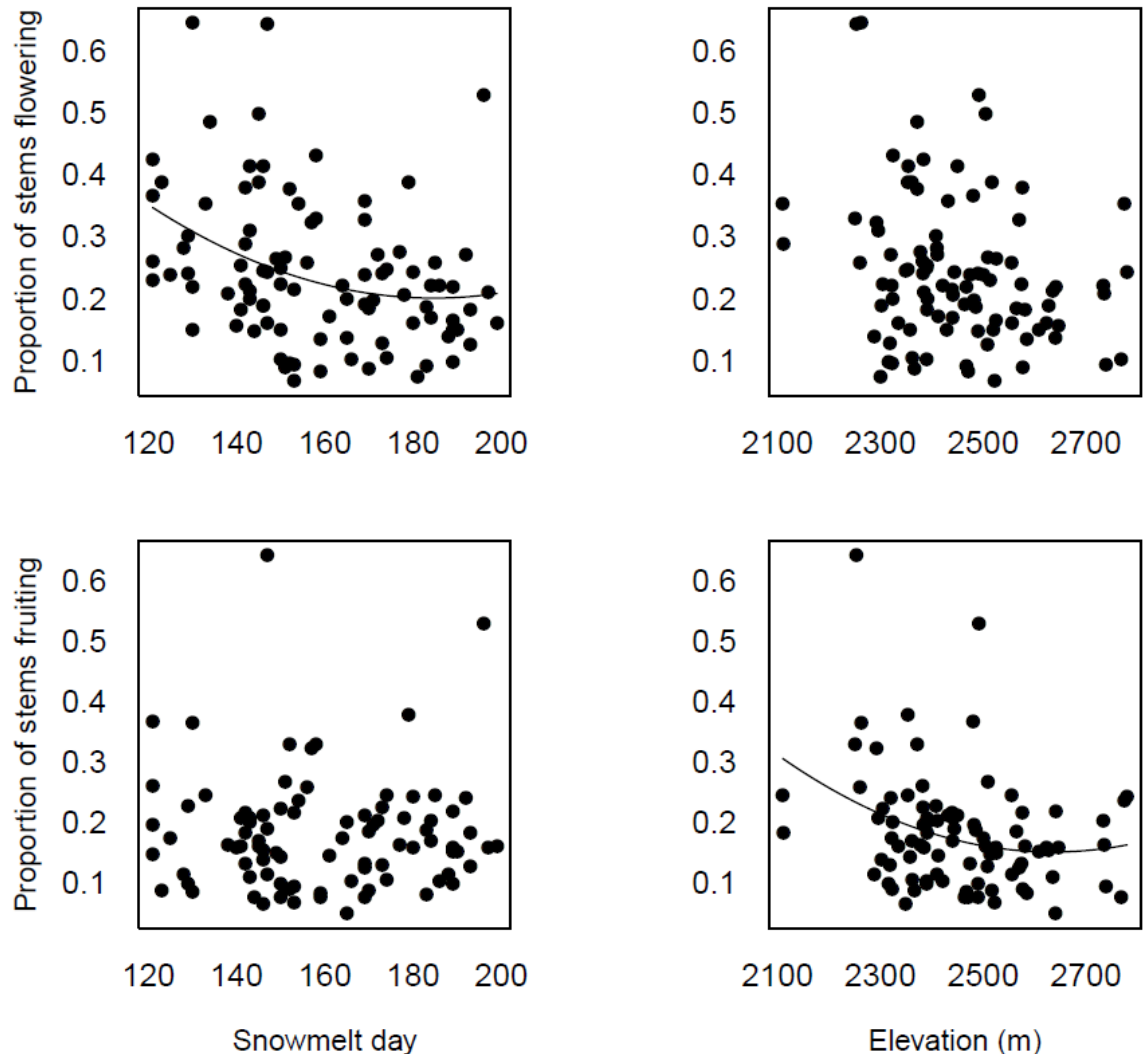


Figure 3: Proportion of stems flowering and fruiting for female *Salix herbacea* averaged for both plot and year along the elevation and snowmelt gradient over three years on three mountains in Davos, Switzerland. Data are fitted with a curve extracted from model estimates when the relationship is statistically significant.

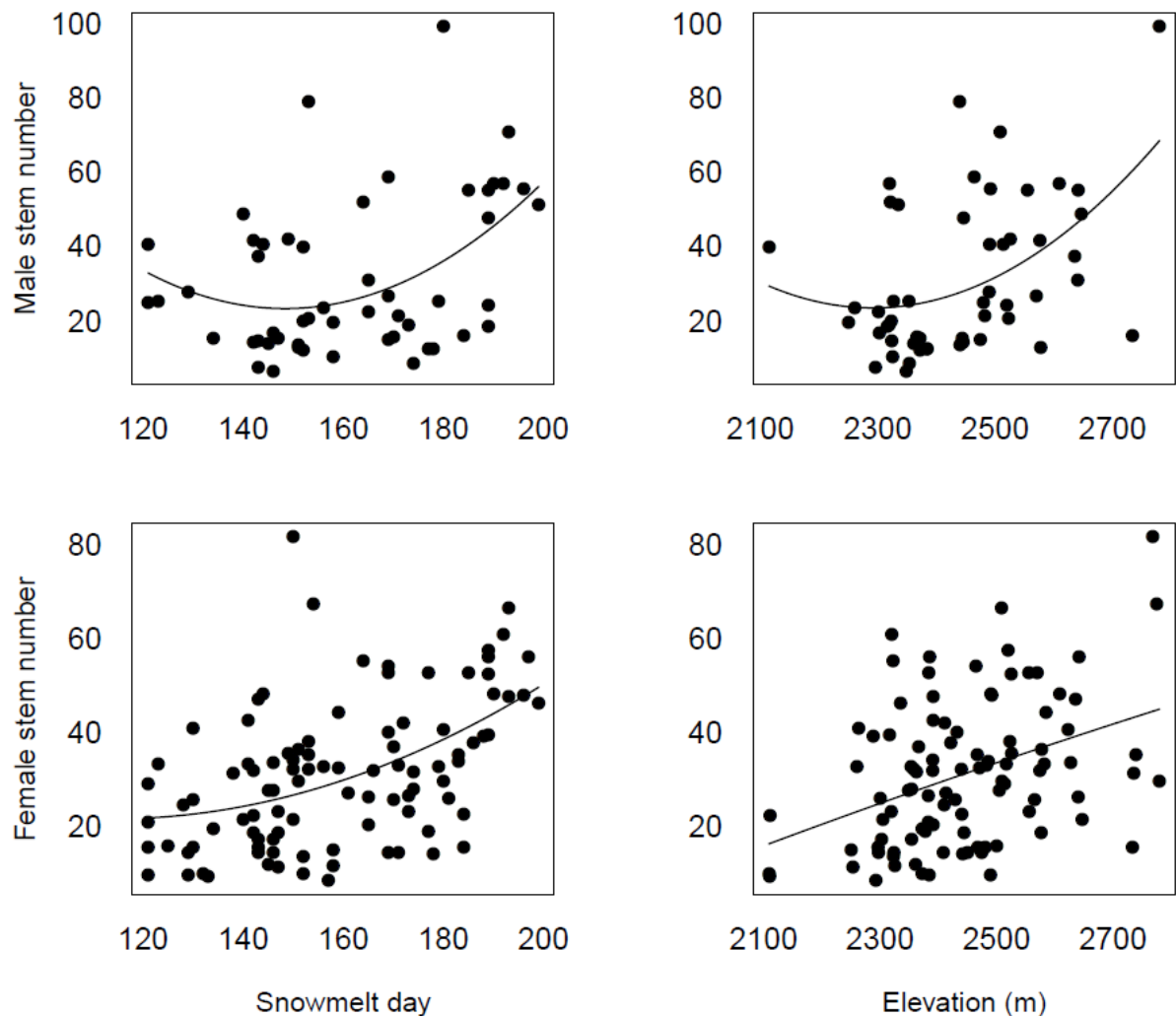


Figure 4: Patch stem density for male and female *Salix herbacea* averaged for both plot and year along the elevation and snowmelt gradient over three years on three mountains in Davos, Switzerland. Data are fitted with a curve extracted from model estimates when the relationship is statistically significant.

## Chapter 3

### **With a little help from my friends: community facilitation for the dwarf shrub *Salix herbacea* under climate change**

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*Salix herbacea* and the ridge shrub community, photo by Anita Nussbaumer

## Abstract

Under climate change, warming and early snowmelt are currently and will continue to alter alpine ecosystems. This may cause a shift between positive and negative plant-plant interaction types, which can affect alpine plant performance and influence community structure. We removed neighbours around *Salix herbacea*, a common arctic and alpine dwarf shrub, in a space-for-time substitution along elevational and snowmelt gradients on three mountains near Davos, Switzerland. The objectives of our study were to determine the effect of neighbours on phenological, morphological and fitness traits of *S. herbacea*, and to determine whether neighbour interactions shift between competition and facilitation along environmental stress gradients.

The majority of traits were controlled primarily by snowmelt timing. However, neighbour removal directly reduced the number of days required for fruit production relative to control shrubs; however, it also increased the likelihood of leaf tissue herbivory. Effects of neighbour removal also changed along the environmental gradients, with neighbour removal



leading to reduced leaf size on earlier snowmelt sites, and increased fungal damage with increasing elevation.

Our results indicate the majority of neighbour interactions influencing *S. herbacea* are facilitative, particularly at stressful early snowmelt and high elevation sites. We suggest neighbours moderate environmental conditions by offering direct protection in the cold early growing season while likely preventing strong radiative warming that may allow increased phytopathogen infection. Further, they may reduce visibility or apparency to leaf herbivores regardless of elevation or microhabitat. We conclude that positive neighbour interactions may be important for the control of multiple traits in *S. herbacea*, and facilitation may become more important under changing climatic conditions, as damage by low temperature events, phytopathogens and leaf herbivores may become more common under early snowmelt.

## Introduction

The effects of climate change have been predicted to be particularly extensive for plant communities in alpine ecosystems (e.g. Parmesan, 2006; Lenoir, Gegout, Marquet, de Ruffray & Brisse 2008). Two major environmental changes, warming temperatures and accelerated spring snowmelt, have been both predicted and observed in alpine tundra habitats (Steger, Kotlarski, Jonas & Schar, 2012; Rixen, Dawes, Wipf, & Hagedorn, 2012). These shifts in environmental conditions can have profound effects on alpine vegetation communities, particularly low-stature shrubs, which are often closely phenologically linked with snowmelt timing (Wipf, Stoeckli & Bebi, 2009).

Low shrubs have been demonstrated to be strongly responsive to both warming and changes in snowmelt timing, but often show mixed responses between species in the same community, or within the range of trait examined for one species. Warming increases biomass production in *Cassiope tetragona* but not in *Salix arctica* occurring in the same communities,

while early snowmelt augments vegetative growth in *Loiseleuria procumbens*, but not in co-occurring *Empetrum nigrum*, *Vaccinium uliginosum* or *V. myrtillus* (Campioli et al., 2013, Wipf, Stoeckli & Bebi, 2009). On a larger community scale, Dorji et al., (2013) and Rumpf, Semenchuk, Dullinger & Cooper (2014) have similarly demonstrated strongly species-specific responses to snowmelt and temperature change in both Arctic tundra and alpine meadows. Within species, warming has been shown to increase flowering in *Arctostaphylos alpina* and *Vaccinium uliginosum* without increasing vegetative growth (Suzuki & Kudo, 2005), and early snowmelt accelerates phenology in many shrub species without a corresponding increase in growth (Wipf, 2010). The inconsistent response of shrubs, both inter-specifically and between traits of the same species, to changes in snowmelt and warming suggests that other local factors represent additional drivers of shrub response to environmental change.

Plants interact with each other within the complex network of their communities, and thus, neighbour interactions may play a key role in determining shrub response to climate change. At the landscape scale, biotic plant-plant interactions can be important for determining the structure of communities, specifically through negative interactions (e.g. resource competition) and positive interactions (facilitation; e.g. Callaway, 2007; Wang et al., 2012, Craine & Dybzinski, 2013). At the species level, interactions with neighbours can directly affect plant fitness traits, both negatively, by reducing fecundity and flower production (Partzsch & Bachmann, 2011), and positively, by increasing survival and growth (Wipf, Rixen & Mulder, 2006). Plant-plant interactions may also mediate other biotic interactions within the community, particularly those involving herbivores, as neighbours have often been shown to reduce herbivory in target species (e.g. Schöb et al., 2010, Louthan Louthan, Doak, Goheen, Palmer & Pringle, 2014). Finally, the stress gradient hypothesis (Bertness & Callaway 1994) predicts that neighbour interactions will shift from mainly competitive to mainly facilitative as environmental stress increases. Thus, neighbour

interactions may be altered or become more important under climate change as habitat stress increases for alpine communities: warming temperatures and earlier snowmelt may lead to drought stress in shallow soils near treeline (Vittoz, Rulence, Largey & Frelechoux, 2008), while earlier exposure from snow increases the likelihood of damaging frost events and herbivory (Roy, Gusewell & Harte, 2004, Inouye, 2008, Wheeler et al., 2014).

Alpine habitats provide excellent natural observatories for environmental stress gradients, and elevational gradients are frequently used to examine shifts in competition and facilitation (e.g. Choler, Michalet, & Callaway, 2001; Jarrad, Walshe, Chee & Burgman, 2012; Grassein, Lavorel & Till-Bottraud, 2014). In addition, alpine ecosystems have highly heterogeneous microtopography and snowmelt timing, and thus can also be used to examine neighbour interactions along snowmelt gradients. Studies examining biotic interactions along snowmelt gradients are rarer (but see Wipf et al., 2006; Schöb et al., 2010), and few if any studies examine biotic interactions along both elevational and snowmelt gradients simultaneously.

*Salix herbacea* L., a common arctic and alpine dwarf shrub, represents an optimal species for studying neighbour interactions along both elevational and snowmelt gradients. In the eastern Swiss Alps, it occurs along a relatively long elevational gradient (~800m) and grows in a wide range of snowmelt microhabitats, from late-lying snowbeds to early-exposure ridges. Past work on *S. herbacea* has indicated that fitness may be reduced and exposure to frost, herbivory and phytopathogen damage will increase under early snowmelt scenarios (Wheeler et al., 2014, Wheeler et al., unpublished). Further, the species has demonstrated plastic responses to changes in snowmelt conditions in several traits, such as leaf size, phenology, and damage likelihood (Sedlacek et al., unpublished). High gene flow has led to low genetic differentiation between populations along elevation gradients and between microhabitats (Cortés et al., 2014), suggesting abiotic environment and plant-plant interactions may strongly contribute to trait differences. Finally, it is a prostrate species that is

only weakly competitive, and is generally restricted to microhabitats where disturbance interferes with the establishment of stronger competitors (Beerling, 1998). Thus, *S. herbacea* represents an excellent candidate to examine the importance of biotic neighbour interactions, and how these interactions may be altered under climate change.

The objectives of this study were thus 1) to examine the direct and indirect effects of neighbour interactions on phenological, morphological and fitness traits in dwarf shrub *S. herbacea*, and when biotic interactions exhibit control over these traits, 2) to determine whether biotic interactions shift from competitive to facilitative along environmental stress gradients, from low to high elevation and from early to late snowmelt. Since *S. herbacea* is a long-lived species, we hypothesize that plant-plant interactions, as demonstrated through neighbour removal, will have the strongest effect on traits that have the potential to respond rapidly to changes in competition or facilitation, such as leaf size. We also hypothesize that neighbour interactions will play a role in herbivory control, and that neighbour removal will increase herbivory in *S. herbacea*. Finally, we predict that biotic interactions will shift from competition to facilitation with increasing elevation and with earlier snowmelt, as environmental stress increases.

## Methods

### *Field sites and experimental design*

We established three study transects near Davos in the eastern Swiss Alps, on Jakobshorn (46°46' N, 09°50' E, 2100 to 2600 m asl), Schwarzhorn (46°44' N; 09°57' E, 2380 to 2780 m asl) and Wannengrat (46°48' N, 09°46' E, 2280 to 2640 m asl). All transects had primarily NE exposure and covered the main elevational range of *S. herbacea* in the area. The Stillberg climate station, established in 1975 at 2090 m a.s.l. at the base of the Jakobshorn transect, has recorded an average annual air temperature of 2.1 °C and annual precipitation of 1150 mm, with snowfall occurring in all months (Rixen et al., 2012). This climate station has

also documented significant climate changes since 1975, with air temperatures in June-July-August increasing on average 0.58°C per decade, and spring snowmelt accelerating by an average of 3.5 days per decade (Rixen et al., 2012).

At ten elevational bands on each of the three transects, we established one ridge microhabitat study plot (c. 3x3 m, early season exposure from snow) and one late snowbed microhabitat plot (late season exposure), for a total of 60 paired plots, relatively evenly spaced along the transects. In each plot after snowmelt in 2011, we selected 3-4 *S. herbacea* patches (10 cm diameter, with intact neighbouring vegetation) as controls, for a final total of 159 patches, and marked them for monitoring. All selected *S. herbacea* patches were separated by 1-2 m, to reduce the probability of resampling the same clone (Stamati, Hollingsworth & Russell, 2007). Then we selected another 10-cm diameter *S. herbacea* patch and clipped all aboveground neighbours in a 40-cm diameter surrounding the patch, for a total of 60 neighbour removal patches. Underground biomass was not removed to minimize disturbance of *S. herbacea* stem and root structures. Neighbour removal patches were situated within 2 m of control patches on all study plots. Neighbour removal patches were then allowed to acclimate through one full growing season (2012), with periodic re-clipping of neighbour biomass as necessary. Due to disturbance from cattle, marmots and rock slides, we report data from 44 neighbour removal patches, and from 159 paired control patches.

### *Data collection*

In 2011, 2012 and 2013, as part of a larger study detailed in Wheeler et al., (unpublished), we visited all control patches weekly, from snowmelt to leaf senescence. We documented snowmelt day, first flowering, and percent damage by insect herbivores and fungal phytopathogens on all control patches. In 2013, after one full season for neighbour removal acclimation, we visited both control and neighbour removal patches weekly from the date of first snowmelt until early September, when the first major snowfall occurred. We

observed the first day of snowmelt, and then each week we recorded leaf status (bud break, expanded), proportion of stems flowering per patch and proportion of stems fruiting per patch, and used these data to estimate the first observation date of each phenological stage (first leaf expansion, flowering, and fruiting). We also recorded leaf tissue damage every week, by estimating the proportion of leaves per patch damaged by insect herbivores and fungi. Vertebrate herbivory was absent for *S. herbacea* in our field sites. For each patch, we recorded mean leaf size (i.e. calculated as an ellipse with the length x width of two undamaged leaves per patch) after full leaf expansion and stem number within the 10-cm patch diameter. We measured soil temperature (5 cm below the surface, 2 hour intervals) for all ridge and snowbed plots using in-situ soil temperature loggers (iButton, Maxim Integrated, San Jose, CA, USA), which were used along with field observations to determine day of snowmelt (the date when soil temperature increased rapidly from the near-0°C temperature that characterizes continuous snow cover).

### *Statistical analyses*

We analysed differences in all traits between neighbour removal patches (44) and controls (159 patches, pooled as mean trait data per study plot) using generalised linear mixed models. We used a normal error distribution for models with phenological development time (period from snowmelt to first day of leaf open, flowering and fruiting phenophases), proportion of stems flowering and fruiting per patch, leaf size and stem number as response variables. Response variables were square-root or log-transformed to ensure normality of residuals when necessary, and standard diagnostic plots were used to confirm assumptions of linearity and homoscedasticity. We used a binomial error distributions for models with fruit set, and the presence/absence of leaf damage (from herbivory and fungi) as response variables. We used snowmelt day, elevation (continuous variables centered to a zero mean to allow appropriate effect estimation; Schielzeth, 2010), and neighbour removal treatment as

fixed terms; the two-way interactions of all fixed terms were included in the models. The nested random factors were study plot, elevational band and transect. Models examining flowering only included data from female plants, as there were too few males in the neighbour removal treatment for analysis.

We analysed relative neighbour effects for each plot containing a neighbour removal patch (44), for all continuous variables that were significantly influenced by neighbour removal x snowmelt or removal x elevation interaction effect. Relative neighbour effects for each plot were calculated as  $RNE = (x_t - x_c) / x$ , where  $x_t$  is the trait value on the nearest control patch,  $x_c$  is the trait value on the paired neighbour removal treatment patch, and  $x$  is the greater of the two values; values are then multiplied by -1 to produce positive RNE values for facilitation and negative values for competition (Callaway et al., 2002). Relative neighbour effects were then analyzed using linear mixed effects models with snowmelt time and elevation as the fixed terms, with transect as the random factor.

To determine if neighbour effects in the previous season influenced fitness (as determined by likelihood of female flowering) in the following year, we used data from a larger, three-year concurrent study, with 480 *S. herbacea* surrounded by intact vegetation. If damage likelihood was influenced by neighbour removal (as detected using the initial mixed models using 44 neighbour removal patches, with damage likelihood influenced either directly, or as an interaction effect with snowmelt time or elevation), we implemented it into a mixed effects model. The response factor was female flowering in the following year (binary, flowers present or absent), with the damage likelihood in the previous season, elevation and snowmelt in the following year as fixed terms, with transect, elevation band and plot as nested random factors. These models were repeated for two years to determine the effect of 2011 damage on 2012 flowering likelihood, and 2012 damage on 2013 flowering likelihood. All statistical analyses were carried out in R v.2.15.1, using package lme4, with p-values generated using MCMC sampling.

The objectives of the study were to examine the effect of neighbour interactions on trait variation along the environmental gradients. Thus, we report and discuss only the direct effects of neighbour removal, and the interaction effects of neighbour removal with snowmelt timing, and with elevation. The direct effects of snowmelt time, elevation and sex on trait variation in *S. herbacea* are discussed extensively in a concurrent study (Wheeler et al., unpublished).

## Results

### *Phenological development time*

Along both elevational and snowmelt gradients, time to leaf expansion and flowering were not significantly affected by neighbour removal treatment (N days from snowmelt to leaf expansion  $\pm$  SE, control mean= 19.7  $\pm$  1.06; neighbour removal mean= 21.6  $\pm$  2.53 and N days from snowmelt to flowering  $\pm$  SE, control mean=22.1  $\pm$  1.17; neighbour removal mean=21.1  $\pm$  2.00). In contrast, there was a significant decrease in time required to develop fruit (N days from snowmelt to fruiting) with neighbour removal (Table 1). Neighbour removal patches produced fruit on average 4.2 days faster than control patches (N days from snowmelt to fruit development  $\pm$  SE, control mean =35.3  $\pm$  1.54, neighbour removal mean = 31.8  $\pm$  2.17). This suggests that neighbour effects on phenological development times are likely small, but tend to increase over time, so that by later phenophases, an effect of neighbour removal becomes observable.

### *Sexual reproduction*

The likelihood of females producing flowers and fruit were high on both treatment types. On the control patches, 80 out of 83 females flowered in 2013, while on neighbour removal treatments, all 21 females flowered. After flowering, 64 females produced fruit on control patches, and 17 females produced fruit on neighbour removals patches. Neighbour



removal had no significant effect on the proportion of flower and fruit production (proportion of stems flowering or fruiting per patch) in female plants neither directly nor along either environmental gradient (i.e. neighbour removal x elevation and neighbour removal x snowmelt; Table 1).

### *Vegetative production*

Neighbour removal had no significant effect on stem number either directly or along either environmental gradient (Table 1). Leaf size, however, was significantly affected by the interaction between neighbour removal and snowmelt time. Leaf size on neighbour removal patches shifted from smaller on early-exposure sites to larger with later snowmelt timing (Table 1, Figure 1A), while leaf size on control patches was relatively similar along the snowmelt gradient (Table 1, Figure 1A). This suggests a facilitative effect of neighbours on leaf size for *S. herbacea* growing on early-exposure sites, and that this interaction shifts to competition with later snowmelt. This was supported by the relative neighbour effects (RNE) on leaf size, which were significantly affected by snowmelt time, and shifted from facilitative (positive) to competitive (negative) with later snowmelt ( $t=2.48$ ; Figure 1B).

### *Likelihood of leaf damage*

The likelihood of both herbivory and fungal damage was influenced by neighbour removal, either directly or with increasing elevation. Likelihood of herbivory damage was significantly higher on neighbour removal patches (over 90%) relative to controls with intact neighbouring vegetation (about 70%; Table 1). Fungal damage likelihood increased with neighbour removal with increasing elevation, whereas fungal damage likelihood on control patches, decreased slightly with increasing elevation (Table 1, Figure 2). These results suggest that neighbours may have a strong protective influence against different damage agents.

Using the 480 plants from the larger, three-year study (all intact vegetation, no neighbour removal treatment), we found both herbivory and fungal damage influenced fitness proxies in female *S. herbacea* in the following year, by reducing flowering likelihood with later snowmelt. This was indicated in the models by an interaction between the previous year's damage likelihood and the snowmelt day in the current flowering season. When damaged by fungi in 2011, females had a decreasing likelihood of flowering with later snowmelt in the following year, whereas undamaged females demonstrated the opposite trend, showing an increasing likelihood of flowering with later snowmelt ( $z=-1.97$ ,  $p=0.049$ ; Figure 3A). When damaged by herbivory in 2012, females had a decreasing likelihood of producing flowers with later snowmelt in the following year, while, again, undamaged females showed an increasing likelihood of producing flowers with later snowmelt ( $z=-2.197$ ,  $p=0.0280$ ; Figure 3B).

## Discussion

Fruiting phenology, leaf size, and leaf damage likelihood by both herbivory and fungi, responded directly to neighbour removal or demonstrated a varied response to neighbour removal along the elevation and snowmelt gradients. Phenological development time from snowmelt to fruiting occurred more rapidly and the likelihood of herbivory was higher with neighbour removal. Leaf size increased with neighbour removal with later snowmelt, demonstrating a shift from facilitation on earlier snowmelt to competition on later snowbeds. Fungal damage became more likely with neighbour removal as elevation increased. Both fungal damage and leaf herbivory were demonstrated to reduce flowering probability in the summer following leaf damage. Thus, the presence of neighbours improves performance in several key traits, particularly on earlier snowmelt sites. Competitive effects of neighbours likely lead to reduced leaf size on later snowmelt sites. While there is likely a trade-off between slower fruit development on one hand and less herbivory and pathogen infection on

the other, most of the observed biotic interactions were facilitative, and may become increasingly important under climate change, particularly under early snowmelt conditions.

#### *Environmental control on trait variation*

While leaf size, fruiting phenology and leaf damage probability were shown to be influenced by neighbour interactions, several key traits were not, most notably sexual reproduction (as measured by flower and fruit production), in addition to stem number, a likely slow-response measure of clonal growth. Our results suggest that these fitness traits in *S. herbacea* may not be strongly influenced by neighbour interactions. Biomass and fecundity have shown a range of responses to neighbour interactions in other studies examining forbs, depending on a wide range of community and landscape factors. For example, while Parkinson, Zabinski and Shaw (2013) found that neighbours did not reduce biomass in a number of sagebrush forb species, Schöb et al. (2010) found neighbour removal increased fruiting and biomass production in alpine snowbed species, although they did not examine *S. herbacea*, despite its common presence in their study sites. Callaway et al. (2011) demonstrated neighbours reduced growth and reproduction in *Centaurea stoebe* within its native range, but that these negative effects did not occur with novel species neighbours in the invaded range of *C. stoebe*.

Since fecundity and growth responses can vary between species and environment types, we suggest *S. herbacea* may either allocate resources differently than the forbs in the examined studies, or alternately, that fitness traits in *S. herbacea* are controlled more strongly by environmental factors, as opposed to plant-plant interactions. In a concurrent study examining trait variation in *S. herbacea*, we examined phenological, morphological and fitness traits over three growing seasons and determined that both flower and fruit production, along with stem number, were driven primarily by snowmelt timing (Wheeler et al., unpublished). We suggested one likely mechanism driving the observed patterns was colder

temperature conditions experienced by plants growing in earlier snowmelt sites, which may lead to increased flower abortion and reduced allocation to stem biomass.

#### *Neighbours shelter S. herbacea against herbivores*

The most direct evidence of facilitation by neighbours was the significant reduction of herbivory likelihood to *S. herbacea* in the presence of intact neighbouring vegetation. Increased herbivory likelihood was observed as a direct response to neighbour removal and was consistent along both snowmelt and elevation gradients. This was similar to Schöb et al. (2010), who found neighbours increased survivorship of snowbed species by protecting them from insect and vertebrate grazers. We suggest three potential mechanisms at work: 1) warmer temperatures after neighbour removal, 2) concentration of herbivores on isolated *S. herbacea* after neighbor removal or 3) higher nutrient availability in *S. herbacea* forage after neighbor removal. First, soil and surface temperatures on neighbour removal sites were likely higher than on sites where neighbour vegetation remained intact, as dark bare soil would absorb more solar radiation than sites with an intact neighbouring canopy. Those warmer bare sites may have been more attractive to insect herbivores, which may prefer feeding on warmer sites (Strauss & Cacho, 2013).

Associational resistance is a mechanism by which plant species may escape or reduce damage sustained from herbivores by growing in close association with neighbouring vegetation. Castagneyrol, Giffard, Pere and Jactel (2013) suggested that associational resistance, derived from the presence of a high-biodiversity neighbouring plant community, is often a function of apparency, where species growing among taller neighbours are less likely to be found by herbivores due to visual and chemical sheltering effects provided by the neighbours. We speculate that apparency may have been an important driver of the observed herbivory pattern, as *S. herbacea* generally represented the lowest vegetation layer in the community. Since the majority of neighbours were taller, neighbour removal likely increased

apparency to insect herbivores. Strauss and Cacho (2013) demonstrated that palatable plant species growing on bare soil sites experienced higher caterpillar herbivory than proximate vegetated sites, and speculated the potential mechanisms as both apparency and herbivore preference for warmer microsites, in addition to herbivore species-specific behaviour of spending more time on isolated plants. Since the primary herbivores for *S. herbacea* were also caterpillars (*Zygaena sp.*), these mechanisms may explain the increased likelihood of herbivory when neighbours are removed.

A final possible explanation for higher herbivory on neighbour removal patches is that the removal of neighbouring vegetation may have increased nutrient availability to *S. herbacea*, and forage with higher nitrogen content has higher palatability (e.g. Torp, Olofsson, Witzell & Baxter, 2010). We have no nutrient data available for our neighbour removal patches, so this mechanism is speculative; however, there was no increased growth or leaf size on neighbour removal patches to suggest a higher nutrient availability.

#### *Facilitation under early snowmelt conditions*

Earlier snowmelt sites may represent stressful microhabitats for *S. herbacea*. In two concurrent studies, we demonstrated that frost damage, herbivory and fungal damage to leaf tissue are all more likely with earlier snowmelt, phenological development is slower, fewer stems are produced, and fruit set declines (Wheeler et al., 2014; Wheeler et al., unpublished). Under stressful early snowmelt conditions, the presence of neighbours facilitates leaf production in *S. herbacea*. Plants produced relatively similar-sized leaves in the presence of neighbours on the control plots along the snowmelt gradient; however, in the absence of neighbours, leaf size was significantly larger in *S. herbacea* growing in later snowmelt sites. This, in addition to the relative neighbour effect analysis, suggests that for leaf size, the primary interaction between *S. herbacea* and its neighbours is facilitative under early snowmelt conditions, and shifts to competition under later snowmelt. Schöb et al., (2010)

demonstrated similar findings, with stronger competition observable between snowbed species on later snowmelt sites, leading to higher biomass on control sites relative to neighbour removal.

This interaction shift supports the main argument of the stress gradient hypothesis (Bertness & Callaway 1994), a shift between facilitative effects on early snowmelt sites, to competition in later snowbeds. Wipf et al. (2006) also showed generally facilitative effects of neighbours on *Empetrum nigrum* with accelerated snowmelt, and suggested the observed facilitation as a response to colder temperatures after exposure from snow. Similarly, Klanderud (2005) demonstrated reductions in *Carex* leaf length with removal of *Dryas octopetala* neighbours in alpine communities in Norway, and also speculated this effect was due to the loss of protection against low temperatures and strong winds. We suggest a similar mechanism at work in our study, as mean soil temperatures in both the first 4 weeks following snowmelt and during the whole growing season are colder for earlier snowmelt sites (Wheeler et al., unpublished), and spring frost events occur for *S. herbacea* almost exclusively on high elevation and early snowmelt sites (Wheeler et al., 2014). Thus in later snowmelt sites, where frost events do not occur, and mean temperatures are warmer, the dominant interaction shifts to competition, and the removal of neighbours results in larger leaves in *S. herbacea*.

Temperature likely also explains the more rapid fruiting phenology on neighbour removal patches relative to controls. Since phenological development in alpine shrubs, including *S. herbacea*, is often driven by temperature accumulation (Wipf et al., 2009; Wheeler et al., unpublished), dark bare soil patches that absorb more solar radiation during the day likely accumulated temperature at a faster rate than vegetated control patches, leading to more rapid fruiting phenology on neighbour removal patches.

#### *Facilitation at high elevations*

The high elevation distribution limits of species are likely controlled by harsh environmental conditions, such as colder mean season temperatures and frost events, including those speculated for *S. herbacea* (Wheeler et al., 2014). Our concurrent study showed plants produce fewer flowering stems and smaller leaves at higher elevations (Wheeler et al., unpublished). At higher elevation, we observed a facilitative effect of neighbours, with a lower likelihood of fungal infection in leaf tissue when *S. herbacea* grows in association with neighbours relative to neighbour removal sites. Higher temperatures have been associated with increased susceptibility to infection by fungal pathogens in crop species (Landa et al., 2006; Siebold & von Tiedemann, 2012). The removal of neighbouring vegetation may have warmed site conditions sufficiently to improve growing conditions for fungal pathogens and in the absence of extensive soil drying, increased the likelihood of fungal infection of leaf tissue. This effect may have been more evident at higher elevation because fungal infection may be more or less absent at colder, higher sites, and neighbour removal may have caused a shift from an absence of fungal infections to a moderate and thus observable fungal infection rate. At warmer, lower elevation sites, this would lead to an increase in an already extant fungal infection rate. An alternative explanation is that the probability of fungal infection may be relatively similar along the elevational gradient, but removal of neighbours leads to higher stress and thus a greater likelihood of infection. In addition, since rust phytopathogen spore dispersal is generally wind-controlled (Helfer, 2014), we suggest that decreased shelter effects after neighbour removal could increase likelihood of fungal infection in exposed *S. herbacea*.

#### *Ecological implications of neighbour interactions under climate change*

Warming temperatures and accelerating spring snowmelt in alpine ecosystems have the potential to alter the biotic neighbour interactions experienced by *S. herbacea*. At higher elevations, temperature increases will likely lead to a more benign habitat, which suggests that

*S. herbacea* may shift to net competition with its neighbours. This may even prompt an upward elevation movement by *S. herbacea* to escape competition. However, under accelerating spring snowmelt conditions, our results suggest facilitative plant interactions may play an important role in the performance and long-term persistence of *S. herbacea*. Indeed, many of the facilitative interactions observed in this study could take on increased importance under changing climatic conditions, particularly the protective influence of neighbours against damage agents.

Tissue damage from herbivory has been shown to increase under warming and earlier snowmelt in alpine ecosystems (Roy et al., 2004; Wheeler et al., unpublished), and can impact plants at both the individual and population levels. Our results show that leaf damage by herbivory can reduce the probability of female flowering with later snowmelt in the year following damage, which would likely reduce potential seed set and thus fitness. Similarly, invertebrate herbivory on leaf tissue has been shown to impact fitness by reducing catkin and seed mass in *Betula pubescens* (Ruohomaki, Haukioja, Repka & Lehtila, 1997). Herbivores have also been shown to control population distributions in grass and forb species by reducing growth, reproduction and biomass production; however, facilitative neighbour interactions have been shown to reduce these herbivory controls (Cushman, Lortie & Christian, 2011; Axelsson & Stenberg 2014). Since low to moderate leaf tissue removal by herbivory was observed to be extremely common for *S. herbacea*, and can influence flowering in the following year, facilitative sheltering effects offered by neighbours throughout the elevational and microhabitat distribution may contribute to a significant performance advantage in sheltered *S. herbacea*. To illustrate, Axelsson and Stenberg (2014) demonstrated that net competition between fireweed and its neighbours represented the dominant interaction type when herbivores were excluded, but when herbivores were present, the dominant biotic interaction switched to net facilitation. We suggest herbivory represents a strong negative selection pressure for *S. herbacea*, since fitness effects were quantifiable following herbivory,



and facilitative effects of neighbours were observed countering herbivory likelihood along both environmental stress gradients in our study, outweighing potential competition.

As a second damage agent, fungal phytopathogen infection rates may also increase in warming climates and under earlier snowmelt (Helfer, 2014; Wheeler et al. unpublished), and have been demonstrated to alter entire tundra communities. Olofsson, Ericson, Torp, Stark and Baxter (2011) showed that fungal pathogen outbreaks, as triggered by changes in climate, can decimate biomass in the dominant species *Empetrum hermaphroditum* and thus dramatically alter ecosystem gross photosynthesis and carbon exchange. Under warming temperature conditions, we speculate that facilitation by neighbours, through thermal control and wind sheltering, may reduce phytopathogen infection likelihood in *S. herbacea*, particularly at high elevations where site conditions may be relatively stressful.

Finally, facilitative effects of neighbours may indirectly increase fitness in *S. herbacea*. Reduced herbivory and phytopathogen damage by neighbor sheltering represents one indirect effect, since both damage agents can reduce flowering probability in later snowbeds in the following season. Another indirect effect is through the production of larger leaves when sheltered by neighbours on early snowmelt sites. Larger leaves have been shown to result in greater flowering probability in *S. herbacea* growing in early-snowmelt sites (Sedlacek et al., unpublished), indicating a local selective pressure for larger leaf sizes in these early-exposure microhabitats. Thus, neighbours may indirectly increase flowering probability and thus fitness, particularly under early snowmelt conditions.

### *Conclusions*

Our study demonstrated that plant-plant interactions can strongly affect the likelihood of leaf damage, may be important for control of plastic traits like leaf size, and that *S. herbacea* may respond differently to the presence of neighbours along elevational and snowmelt gradients. The majority of neighbour interactions experienced by *S. herbacea* were

facilitative, particularly at the more stressful extremes of their environmental gradients, supporting the stress gradient hypothesis. Neighbours directly reduce the proportion of herbivory damage and help shelter *S. herbacea* from fungal damage at higher elevations. By reducing damage, neighbours could indirectly improve plant fitness, as damage can lead to female flowering reductions in the following growing seasons. The presence of neighbours also facilitates the production of larger leaf sizes under environmentally stressful early snowmelt conditions. Under accelerated snowmelt conditions in alpine ecosystems, we speculate that shifts to facilitative interactions may become more common as early snowmelt can lead to stressful conditions for *S. herbacea*. Further, facilitative effects of neighbours may become more ecologically important, as they may potentially serve to increase leaf sizes and reduce leaf tissue loss to herbivores and phytopathogens, which may become more common and thus more damaging under early snowmelt conditions.

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Table 1: Responses of *Salix herbacea* functional traits to neighbour removal (NR) treatment and the two-way interaction effects between neighbour removal x snowmelt time and neighbour removal x elevation two years after neighbour removal on three mountains in Davos, Switzerland. Model estimates, t- or z-values and p-values were generated using general and generalized linear mixed models. Significant p-values are bolded.

Response trait	Neighbour removal treatment			Elevation* NR treatment			Snowmelt* NR treatment		
	Estimate	t / z value	p value	Estimate	t / z value	p value	Estimate	t / z value	p value
Time to leaf expansion	-0.011	-0.20	0.84	0.097	1.74	0.083	-0.083	-1.53	0.13
Time to flowering	0.022	0.34	0.73	0.023	0.30	0.76	-0.022	-0.33	0.74
Time to fruiting	-0.14	-2.37	<b>0.021</b>	0.090	1.51	0.14	-0.038	-0.68	0.50
Proportion stems flowering	-0.072	-1.41	0.16	0.046	0.76	0.44	0.096	1.75	0.084
Proportion stems fruiting	0.0017	0.007	0.99	-0.17	-0.71	0.47	-0.14	-0.62	0.54
Fruit set likelihood	-1.28	-1.30	0.19	-0.048	-0.043	0.97	-1.94	-1.85	0.065
Leaf size	-0.37	-1.40	0.16	0.26	0.93	0.36	0.52	2.00	<b>0.048</b>
Stem number	-0.069	-0.22	0.83	0.047	0.15	0.88	0.22	0.70	0.49
Herbivory damage likelihood	1.96	2.22	<b>0.027</b>	-0.34	-0.50	0.62	-0.11	-0.12	0.90
Fungal damage likelihood	0.55	1.29	0.20	0.84	1.99	<b>0.047</b>	-0.44	-0.90	0.37

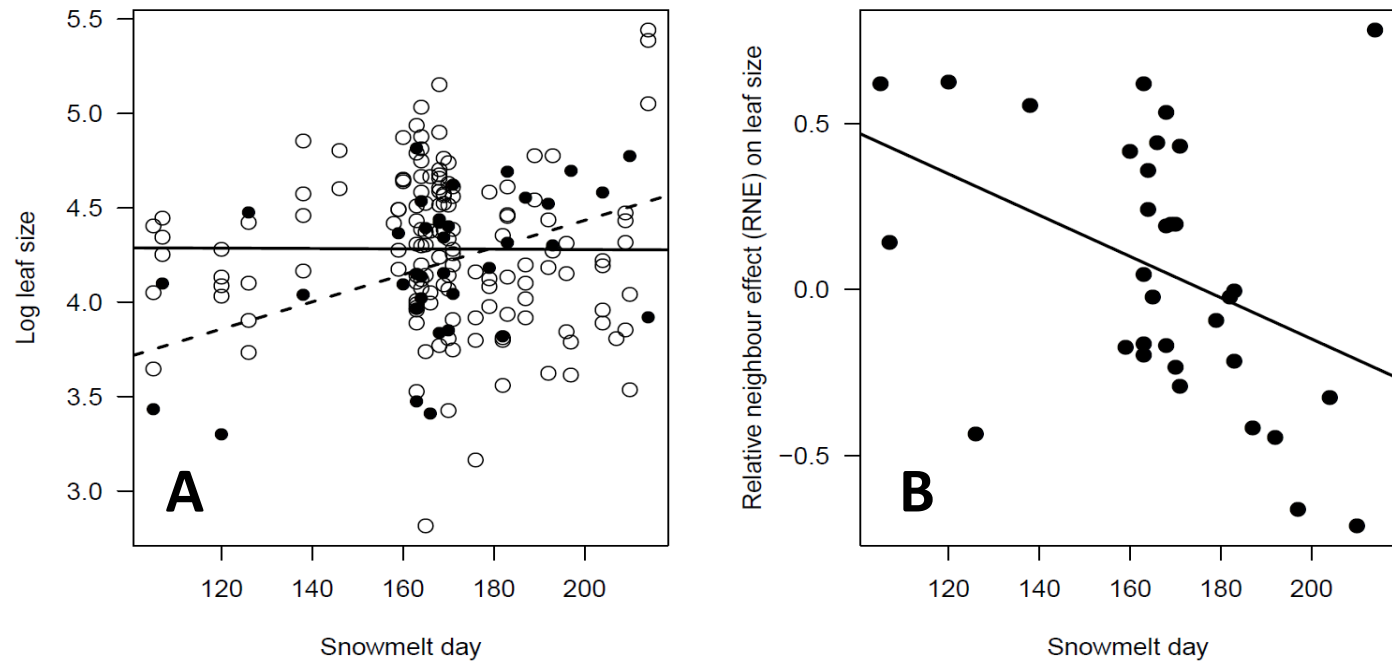


Figure 1: Effects of neighbour removal on leaf size in *Salix herbacea* along snowmelt timing gradients on three mountains near Davos, Switzerland in 2013. Panel A shows log leaf size ( $\text{mm}^2$ ) two years after neighbour removal treatment (dashed line, black points) and on control patches (solid line, white points) and Panel B shows relative neighbour effects (RNE) on leaf size ( $\text{mm}^2$ ) along the snowmelt gradient, where positive values indicate facilitative neighbour effect, and negative values indicate competitive neighbour effect.

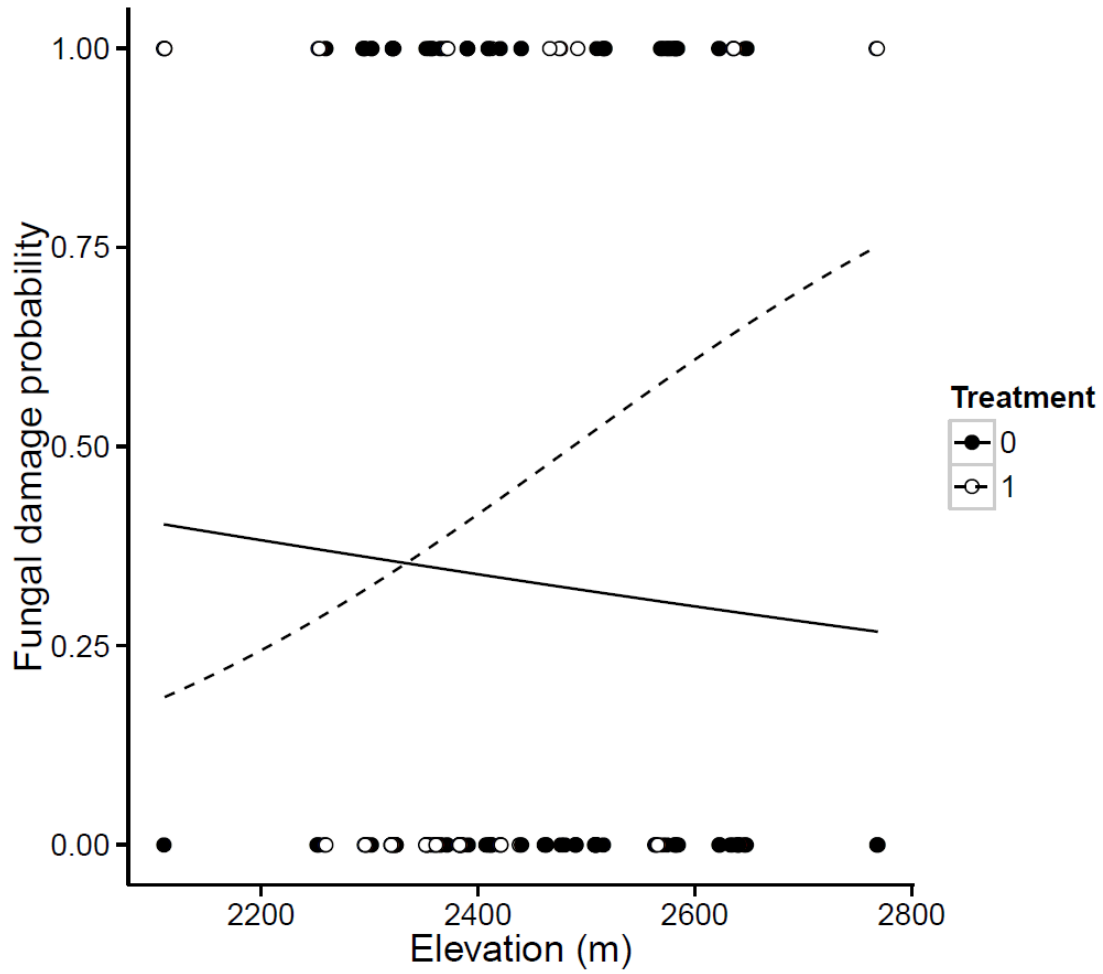


Figure 2: Effects of neighbour removal on leaf damage probability in *Salix herbacea* with fungal damage likelihood on neighbour removal (Treatment=1, white points, dashed line) and on control patches (Treatment=0, black points, solid line) along elevational gradients on three mountains near Davos, Switzerland, in 2013 two years after neighbour removal treatment.

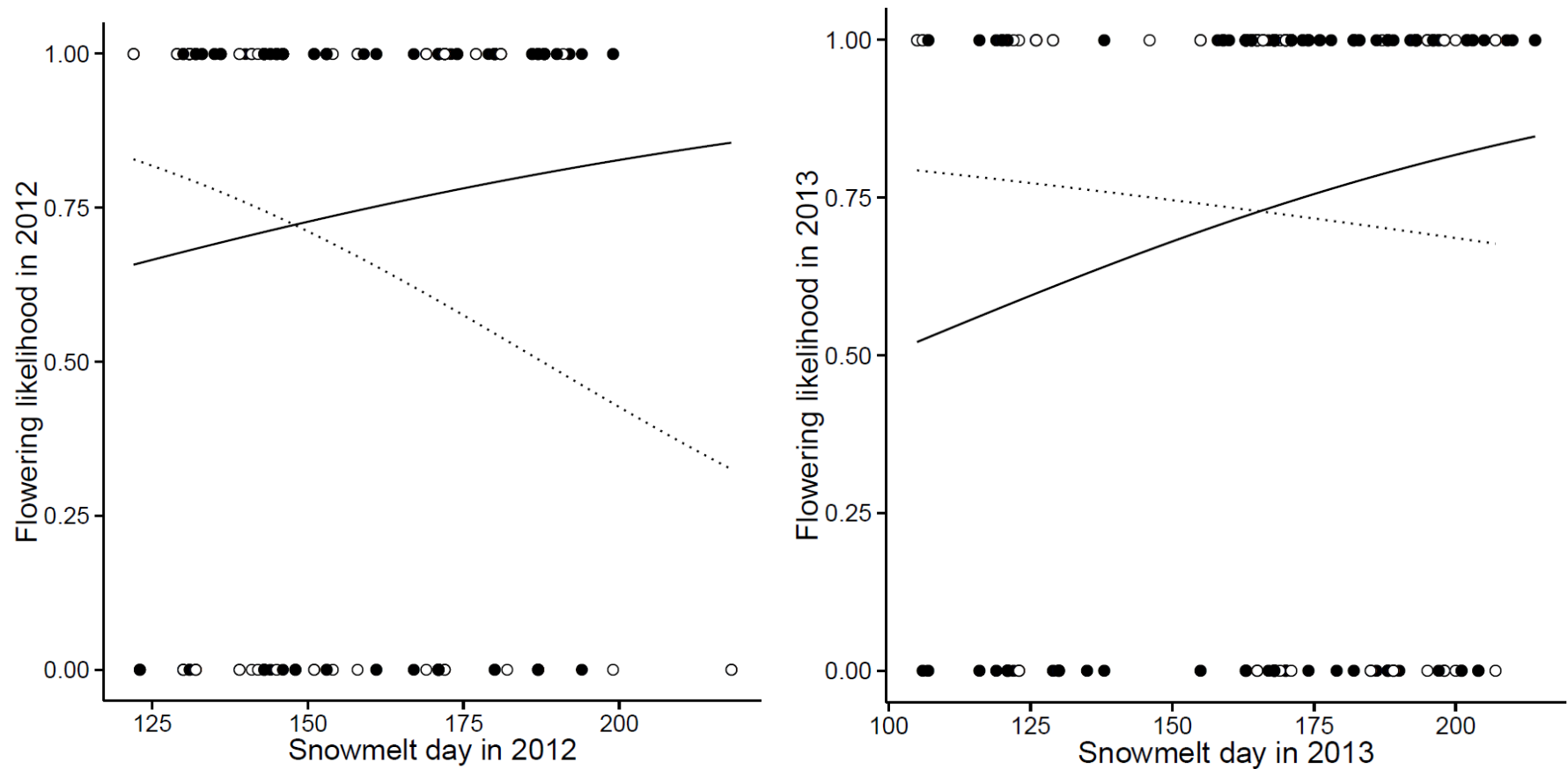


Figure 3: Effects of fungal damage (left panel) and herbivory damage (right panel) on flowering probability in female *Salix herbacea* in the following growing season along the snowmelt gradient on three mountains near Davos, Switzerland; black points, solid line show flowering probability of previously undamaged plants and white points, dashed line show flowering probability of plants damaged by A) fungi and B) herbivory in the previous season.

## Chapter 4

### **The response of the alpine dwarf shrub *Salix herbacea* to altered snowmelt timing: lessons from a multi-site transplant experiment**

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Janosch Sedlacek, Julia Wheeler, Andrés J. Cortés, Oliver Bossdorf, Guenter Hoch, Christian Lexer, Sonja Wipf, Sophie Karrenberg, Mark van Kleunen, Christian Rixen. The response of the alpine dwarf shrub *Salix herbacea* to altered snowmelt timing: lessons from a multi-site transplant experiment. In review, *Plos One*.



Female *Salix herbacea* releasing seeds, Photo by Janosch Sedlacek

## Abstract

Climate change is altering spring snowmelt patterns in alpine and arctic ecosystems, and these changes may then alter plant phenology, growth and reproduction. To predict how alpine plants respond to shifts in snowmelt timing, we need to understand trait plasticity and its effects on growth and reproduction, and the degree to which plants experience a home-site advantage. We tested how the common, long-lived dwarf shrub *Salix herbacea* responded to changing spring snowmelt time by reciprocally transplanting turfs of *S. herbacea* between early-exposure ridge and late-exposure snowbed microhabitats. After the transplant, we monitored phenological, morphological and fitness traits, as well as leaf damage, during two growing seasons.

*Salix herbacea* leafed out earlier, but had a longer development time and produced smaller leaves on ridges relative to snowbeds. Phenological changes did not influence clonal or sexual reproduction, but smaller leaves on ridges were associated with reduced sexual reproduction. Clonal and sexual reproduction showed no response to altered snowmelt time.



We found no home-site advantage in terms of sexual and clonal reproduction. Leaf damage probability depended on snowmelt and thus exposure period, but had no short-term effect on fitness traits.

We conclude that the studied populations of *S. herbacea* can respond to shifts in snowmelt by plastic changes in phenology and leaf size, while maintaining levels of clonal and sexual reproduction. The lack of a home-site advantage suggests that *S. herbacea* may not be adapted to different microhabitats. The studied populations are thus unlikely to react to climate change by rapid adaptation, but their responses will also not be constrained by small-scale local adaptation. In the short term, snowbed plants may persist due to high stem densities. However, in the long term, reduction in leaf size and flowering, and increased exposure to damage may decrease overall performance of *S. herbacea* under earlier snowmelt.

## **Introduction**

Temperature, precipitation and, as a consequence, snowmelt patterns in alpine ecosystems are changing dramatically, with observations of snowmelt acceleration in spring [Rixen et al. 2012]. Model simulations predicting further declines of snow-cover duration with 30-80% in the Alps by the end of the century [Steger et al. 2012]. Since the strongest snow-cover reductions are predicted for spring [Steger et al. 2012], earlier snowmelt may prolong the growing season above 2000 m asl by up to 60 days [Beniston et al. 2003]. A longer growing season could have strong impacts on plants, as it may alter the timing of phenological development, increase exposure to frost, change moisture availability and alter interactions with pollinators, herbivores and pathogens [ Beniston et al. 2003, Wheeler et al. 2014, Wipf et al. 2009, Inouye 2008, Roy et al. 2004, Høye et al. 2013, Little 2014]. However, potential impacts have been assessed for only a few alpine species.

To respond to changing snowmelt conditions, alpine shrubs must either track their climate requirements by migrating to sites with suitable microclimates, or persist under the

new conditions through phenotypic plasticity or adaptive evolution [Bazzaz 1996, Theurillat and Guisan 2001, Bellard et al. 2012]. However, the potential for migration might be limited in species with long generation times [Aitken et al. 2008] and poor dispersal ability [Midgley et al. 2006], and in species growing in fragmented landscapes [Jump and Penuelas 2005]. Recent studies have also considered how shifts in biotic interactions limit migration potential, including changes in herbivory and pathogen damage [Rasman et al. 2014, Van Grunsven et al. 2007, Castro et al. 2010, Defosse et al. 2011, Van der Putten et al. 2013, Sedlacek et al. 2014].

If migration potential is limited, the only way plants can respond to change is by adjusting to the new environmental conditions. In the short term this can be achieved through plasticity, potentially mediated by high clonal and sexual reproductive rates [Williams et al. 2008]. In the long term, adjustment to climate change can take place through adaptive evolution [Bradshaw and Holzapfel 2006, Skelly et al. 2010, Franks et al. 2014]. The latter requires both the presence of genetic variation in relevant traits, and selection acting on these traits. If local adaptation is detected in spite of ongoing gene flow, this suggests strong selective forces [Kawecki and Ebert 2004]. Adaptive evolution, however, might be too slow to keep pace with environmental change, especially in long-lived alpine species [Jump and Penuelas 2005, Franks et al. 2014]. Phenotypic plasticity, in contrast, allows plants to rapidly adjust to changing environmental conditions within the lifetime of a species, and thus may play a key role in species responses to climate change, particularly in long-lived species. Phenotypic plasticity has been mainly demonstrated for phenological changes (e.g. in timing of bud burst) in response to warmer temperatures [Kramer 1995, Anderson et al. 2012]. However, phenotypic change could reflect passive plastic responses as a consequence of resource limitation. Such a passive plastic response may be neutral or even maladaptive [van Kleunen and Fischer 2005, Nicotra et al. 2010], if it does not benefit fitness [Scheepens and Stöcklin 2013, Kim and Donahue 2013]. It is therefore important to understand whether

plasticity allows a species to respond to climate change [Merilä and Hendry 2014], or whether adaptive responses or shorter-distance migration would be needed for regional persistence.

In order to rigorously test how plants respond to snowmelt change through phenotypic plasticity, as well as whether plants experience a home-site advantage, reciprocal transplant experiments are necessary [Kawecki and Ebert 2004, Blanquart et al. 2013]. Many previous studies have used such transplant experiments to investigate phenotypic plasticity and local adaptation in alpine plant species across altitudinal gradients [Frei et al. 2013, Byars et al. 2007, Gonzalo-Turpin and Hazard 2009]. However, reciprocal transplant studies explicitly examining the effects of altered snowmelt timing are scarce (but see [Bennington and Fetcher 2012, Stinson 2004, Kawai and Kudo 2011]). Further, almost all previous transplant experiments examined short-lived perennial herbs, and experiments with long-lived woody species are extremely rare (but see [Bennington and Fetcher 2012]). However, it is important to understand how long-lived species will respond to changes in snowmelt timing, as they are a dominant functional type in alpine plant communities.

The long-lived, arctic-alpine dwarf shrub *Salix herbacea* typically occurs in late-snowmelt microhabitats but also on wind-exposed mountain ridges [Beerling 1998]. A concurrent 3-year observational study at the same mountain, which included a microhabitat and elevation gradient, indicated that most traits of *S. herbacea* were affected mainly by snowmelt microhabitat type (Wheeler et al., unpublished). These microhabitats are mainly differentiated by the duration of winter snow cover [Schöb et al. 2008], but also by environmental factors closely linked to or controlled by snowmelt timing, such as temperature, soil moisture, and biotic interactions, which are predicted to change due to climate change. Therefore, this natural microhabitat setup is well-suited to test the effects of climate change-driven shifts in snowmelt timing in a reciprocal transplant experiment.

In order to make predictions about the potential responses of *S. herbacea* to changes in snowmelt timing, we must understand to what degree phenotypic plasticity can help to adapt

to the new environmental conditions, and whether the species exhibits a home-site advantage in different microhabitats. To this end, we reciprocally transplanted turfs of *S. herbacea* between early-exposed ridge and late-exposed snowbed microhabitats. We investigated the effects of both origin and destination microhabitats on morphological, phenological and fitness traits of *S. herbacea*, assessed leaf damage, and asked the following questions:

1. Does altered snowmelt timing lead to plastic responses in phenology, leaf size, clonal reproduction and sexual reproduction?
2. Does altered snowmelt timing lead to a change in leaf-damage probability?
3. Does *S. herbacea* demonstrate a home-site advantage to local microhabitat snowmelt conditions, suggesting local adaptation?
4. How do phenology, leaf size and damage affect flowering and clonal reproduction in the different microhabitats?

## Methods

### *Study species*

*Salix herbacea* L. is a long-lived, clonal, dioecious, prostrate dwarf shrub, occurring in the northern and alpine regions of Eurasia and North America, and the Arctic region [Beerling 1998]. The species produces an extensive ramifying system with branched rhizomes forming flat mats [Beerling 1998]. The aerial branches are woody and usually reach 2-5 cm above the ground surface. *Salix herbacea* is characteristic to a wide range of microhabitat types, from ridge and scree habitats with early exposure from snow in spring and relatively long growing seasons, to snowbeds with long snow cover duration and short growing seasons. Ridges in our study area were dominated by the shrubs *Loiseleuria procumbens* and *Vaccinium uliginosum*, in addition to the herb *Phyteuma hemisphaericum*. Snowbeds were characterized by the herb *Gnaphalium supinum* and the moss *Polytrichastrum sexangulare*.

### *Reciprocal transplant experiment*

To test for plastic responses and potential adaptation of *S. herbacea* to joint changes in snowmelt patterns and temperature, we established a reciprocal transplant experiment with six pairs of sites. Each pair consisted of an early-exposed ridge site and a nearby late-exposed snowbed site (see Figure A1 and Table A1, for site characteristics). Snowmelt on ridges occurred on average one month earlier than in snowbeds (mean  $\pm$  SE Julian day of snowmelt; ridge:  $157 \pm 3.6$ , snowbed:  $191 \pm 3.1$ ) across both years 2012 and 2013, and earlier in 2012 than in 2013 (2012:  $170 \pm 7.2$ , 2013:  $179 \pm 4.4$ ). As a consequence of earlier snow disappearance and exposure to colder spring temperatures, ridges were colder when averaged over the entire growing season compared to snowbeds (mean  $\pm$  SE of temperature from snowmelt to end of growing season (Julian day 217; ridge:  $10.89 \pm 0.44^\circ\text{C}$ , snowbed:  $11.88 \pm 0.50^\circ\text{C}$ ). Despite differences in timing of snowmelt, the total growing degrees days per season (sum of growing degree days with a threshold of  $5^\circ\text{C}$ , between snowmelt and Julian day 217; GDD) were similar on ridges and in snowbeds (mean  $\pm$  SE of GDD; ridge:  $974.05 \pm 106.17^\circ\text{C}$ , snowbed:  $922.23 \pm 71.35^\circ\text{C}$ , Figure A2). We assessed only snowmelt timing and temperature in our microhabitats, because they are the most prominent aspects of climate change in the Alps, and are generally key regulators of other abiotic and biotic conditions, like soil moisture, nutrient availability, freezing events, and vegetation cover and composition [Wipf et al. 2009, Theurillat and Guisan 2001, Schöb et al. 2008].

The 12 sites were located at the same altitude (2320 – 2355 m asl) on a northeast-facing slope near Davos in the eastern Swiss Alps (Table A1). The distance between two sites in a pair ranged from 28 to 55 m, and the distance between pairs of sites ranged from 40 to 190 m. Within each site, we haphazardly selected and excavated 14 *S. herbacea*-containing soil patches with a diameter of 10 cm and a soil depth of 5 cm. To reduce the probability of sampling the same genotype multiple times [Hägberg 2014], we chose patches that were at least 1 m apart and had no visible connections. We carefully cut each patch into two halves

(turfs), ensuring that each turf contained a minimum of five *S. herbacea* stems. We then transplanted one turf back in the same site (“home site”), and transplanted the other turf in the other microhabitat of the site pair (“away site”). Overall, we transplanted 336 turfs (12 sites × 14 patches × 2 turfs). We minimized negative effects of the transplant on plant performance by maintaining large root systems for each turf and by transplanting at the end of the growing season (15th-16th August 2011), after terminal buds had already formed. Since the first two weeks after transplanting were dry, we watered all turfs twice during these two weeks. We used turfs in our reciprocal transplant instead of seeds or seedlings as seed germination in the field has been observed to be extremely low and progress from the seedling stage to sexual maturity is expected to be very slow, as is typical for slow-growing clonal woody shrubs.

#### *Data collection*

At each site, we recorded soil temperature every 2 hours at 5 cm soil depth using in-situ temperature loggers (iButton, Maxim Integrated, San Jose, CA, USA). We used this temperature data, in conjunction with field observations to determine the day of snowmelt for each site as the date when soil temperature rose abruptly from near 0°C (which is the characteristic soil temperature below snow cover in spring).

We assessed a set of phenological, morphological and fitness traits, which have been suggested to be key plant traits for the assessment of plastic responses to climate change [Nicotra et al. 2010]. At transplanting, we counted the number of stems on each turf, and we used this number as a proxy for initial plant size. Over the two growing seasons (2012 and 2013) following transplant, we weekly monitored phenology, proportion of flowering and fruiting stems, and whether any leaves were damaged by herbivores, fungi or gall-forming insects. The development time to each phenophase (leaf expansion, flowering, fruiting) was calculated as the difference from day of snowmelt until onset of the respective phenophase. Development times to each phenophase were strongly correlated with each other (leaf

expansion and flowering:  $r = 0.78$ ,  $P < 0.001$ ,  $n = 278$ ; leaf expansion and fruiting:  $r = 0.80$ ,  $P < 0.001$ ,  $n = 123$ ; flowering and fruiting:  $r = 0.81$ ,  $P < 0.001$ ,  $n = 113$ ), so onset of and phenological development time until leaf expansion are discussed as representative phenological variables from this point onwards (see Table A2 for results for flowering and fruiting). At leaf maturity, we assessed individual leaf size (i.e.  $\pi \times \frac{1}{2}$  length  $\times \frac{1}{2}$  width taken from two undamaged, fully-expanded leaves per turf, that were selected haphazardly at the terminal point of two stems) and stem number per turf. The net relative change in stem number between 2012 and 2013 was calculated as the ratio of stem number in 2013 to stem number in 2012. So, values larger than one would indicate a net increase, while values smaller than one would indicate a net decrease.

No specific permits were required for the study location and activities, and the field studies did not involve endangered or protected species.

### *Statistical Analysis*

We used generalized linear models to test whether phenotypic variation was explained by the microhabitat where the turfs were planted (i.e. destination effect, which would indicate phenotypic plasticity), by their site of origin (i.e. origin effect, which would indicate genetic effects or environmental carry-over effects), or an interaction of both (i.e. destination  $\times$  origin effect, which could indicate a home-site advantage or disadvantage). The model was designed as follows : Trait  $\sim$  Origin + Destination + Origin:Destination, random = (1|Turf/Patch/Site/Pair).

The response variables leaf size, and onset of and phenological development time to leaf expansion were analyzed with a Normal error distribution. Leaf size was log-transformed to achieve normality and homoscedasticity of the residuals. For stem number, we used a Poisson error distribution, and for the proportion of flowering and fruiting stems and the presence of leaf damage, we used a binomial error distribution. For each response variable,

the models included microhabitat of origin (ridge vs. snowbed), microhabitat of destination (ridge vs. snowbed), year (2012 and 2013) and their interactions as fixed effects. We considered turf nested within patch, nested within destination site, nested within pair as random effects, to account for non-independence of measurements repeated on the same turfs in different years, and non-independence of patches from the same sites within pairs. Proportions of flowering stems were analyzed only for 2013, because flower buds in 2012 were pre-formed in the year before the transplant. Fruit set was excluded from the analysis, due to the low number of fruiting plants in 2013 ( $N < 25$  turfs). In these analyses, with only a single measurement per turf, we excluded turf as a random effect. To test whether the proportion of flowering stems depended on the sex of the plant, we added sex as an additional fixed factor, as well as interactions between sex and microhabitat of origin and microhabitat of destination. To account for differences in stem number at transplant, we included initial stem number (from 2011) as a covariate in the model for stem number.

To test whether phenology and leaf size were under selection in the ridge and snowbed sites, and to determine whether leaf damage impacted clonal and sexual reproduction, we used selection-gradient analyses [Lande and Arnold 1983]. The flowering probability and the net change in stem number between 2012 and 2013, which includes both the growth of new stems and stem die-off was used as a proxy for sexual and clonal reproductive fitness, respectively. We regressed the standardized change in stem number and flowering probability on leaf size, measured in the previous year, for phenological development time (duration from snow disappearance to leaf expansion) and damage probability, for ridge and snowbed destinations separately. For the change in stem number, we used a Normal error distribution, and for flowering probability, we used a binomial error distribution. Because flowering probability is influenced by stem number, we included stem number as an additional explanatory variable. We included site as random effect, to account for non-independence of turfs measured in the same site.



We used log-likelihood-ratio tests to determine the overall significance of main effects and interactions [Zuur 2009]. We also calculated marginal  $R^2$  (proportion of variance explained by the fixed effect only) and conditional  $R^2$  (proportion of variance explained by both the fixed and random factors) as a measure of goodness-of-fit of each model [Nakagawa and Schielzeth 2013]. All statistical analyses were performed using linear and generalized linear mixed models as implemented in the *lmer* function of the lme4 package [Bates et al. 2013] in R version 2.15.2 [R Core Team 2013].

## Results

### *Clonal and sexual reproduction*

The number of stems was significantly higher for turfs originating from snowbeds than for those from ridges (Table 1). The difference in number of stems increased over time depending on turf origin (Table 1, Fig. 1a). We did not find a significant destination-by-origin interaction. Overall, these results indicate that initial differences in stem number between origins did not disappear but even became larger during the course of the study, and that plants from snowbeds did not see significant reductions in stem number when transplanted to ridges.

Turfs originating from ridges had on average a greater proportion of flowering stems than turfs from snowbeds (Table 1, Fig. 2), and ridge and snowbed turfs produced a similar absolute number of flowering stems (ridge turfs:  $2.60 \pm 0.38$ , snowbed turfs:  $2.55 \pm 0.37$ ). The origin effect was evident for both sexes, and present in both microhabitats (Table 1). We found no destination-by-origin interaction and thus no indication for a home-site advantage using proportion of flowering stems as fitness trait. Further, there was a marginally significant interaction effect between sex and destination ( $P = 0.061$ , Table 1), with female plants producing a greater proportion of flowering stems on ridges relative to snowbeds, whereas males were flowering similarly in both microhabitats.

### *Phenology*

Onset of leaf expansion and time from disappearance of snow to leaf expansion was significantly influenced by microhabitat of destination, with turfs in snowbeds expanding their leaves later and requiring less time for this after snowmelt relative to ridges (Table 1, Fig. 1c). Both onset of and phenological development time to leaf expansion were not influenced by origin, but onset of leaf expansion was influenced by year and a year x destination interaction, due to a very early snowmelt in 2012. As other phenological stages were strongly correlated (see Methods), this suggests that both onset of the phenophase and the phenological development time are controlled by the destination environment in a similar way for plants originating from snowbeds and ridges, and thus respond plastically to environmental differences between the microhabitats. Selection gradient analysis showed that development time to leaf expansion did not affect the change in stem number nor the probability of flowering in both ridge and snowbed destinations, indicating that development time required for leaf expansion does not affect sexual and clonal reproduction, at least in the short-term (Fig. 3a,b; ratio of stem number: ridge: d.f. = 1,  $\chi^2 = 0.017$ ,  $P = 0.849$ ; snowbed: d.f. = 1,  $\chi^2 = 0.280$ ,  $P = 0.596$ ; flowering probability: ridge: d.f. = 1,  $\chi^2 = 0.529$ ,  $P = 0.466$ ; snowbed: d.f. = 1,  $\chi^2 = 0.208$ ,  $P = 0.648$ ).

### *Leaf size*

Leaf size strongly differed between microhabitats of destination and between years. Shrubs produced larger leaves in snowbeds than on ridges (Table 1). This destination-site effect was consistent across years, but overall leaves were larger in 2013 than in 2012 (Table 1). There were no significant interactions among origin, destination and year. These results suggest that leaf size is a highly plastic trait, responding to environmental differences between the microhabitats and between years. Selection gradient analyses indicates that leaf size in 2012

did not affect the change in stem number from 2012 to 2013, neither in the ridge nor in the snowbed destination, indicating that a plastic response in leaf size does not affect clonal reproduction in either microhabitat (Fig. 3d, ridge: d.f. = 1,  $\chi^2 = 1.323$ ,  $P = 0.249$ ; snowbed: d.f. = 1,  $\chi^2 = 0.643$ ,  $P = 0.422$ ). However, selection gradient analysis demonstrated that smaller leaf sizes resulted in a reduced probability of flowering on ridges (Fig. 3c, d.f. = 1  $\chi^2 = 5.392$ ,  $P = 0.020$ ).

### *Leaf damage*

The probability of leaf damage by herbivores and pathogens was significantly affected by destination, and the magnitude of this effect changed between years (i.e. there was a significant destination by year interaction, Table 1). Damage probability was higher on ridges than in snowbeds (Table 1, Fig. 1d), and while this effect was consistent across years, it was overall much stronger in 2012 than in 2013. Origin of turfs did not affect damage probability, suggesting that all shrubs were equally vulnerable when exposed to damage agents on ridges. Selection gradient analysis showed that damage did not affect clonal or sexual reproduction in neither of the microhabitats in the short-term (Fig. 3 e, f).

## **Discussion**

In our study, *S. herbacea* demonstrated evidence of both trait plasticity and microhabitat origin effects. *Salix herbacea* turfs that were transplanted between early and late snowmelt microhabitats exhibited a rapid plastic response to the change in microhabitat for both phenological development and leaf size. In contrast, flower production and stem number were only affected by the microhabitat of origin, at least during the two years of our study *S. herbacea* demonstrated no evidence of a home-site advantage for any of the measured fitness traits. Increasing leaf size was associated with an increase in flowering probability on ridges in the selection gradient analysis. Plants were more likely to incur leaf damage by herbivores

and pathogens on ridges than in snowbeds. This suggests that although the studied populations of *S. herbacea* can respond plastically to earlier snowmelt by adjusting phenological and morphological traits, exposure to leaf damage agents becomes more likely. Further, passive plastic reduction in leaf size with earlier snowmelt may lead to reduced flowering, thereby lowering fitness.

#### *Microhabitat origin and destination effects*

Phenology responded strongly to changes in snowmelt timing. Turfs on ridges started earlier but developed more slowly to leaf expansion, flowering and fruiting, while turfs in snowbeds started later but developed faster. Phenology in dwarf shrubs is often closely linked to snowmelt timing, with accelerated snowmelt leading to earlier phenological start in many arctic and alpine species [Wipf et al. 2009, Wipf 2010]. In a field survey on *S. herbacea*, Wheeler et al. (unpublished) found similar growing-degrees days (GDD) accumulation thresholds required for starting leaf expansion and flowering along both elevation and snowmelt-timing gradients. Similarly, many arctic and alpine plants must reach GDD temperature accumulation thresholds in order to move to the next phenophase [Galen and Stanton 1993, Kudo and Suzuki 1999, Huelber et al. 2006, Molau 2005]. Thus, the mechanism driving the starting time and progression of *S. herbacea* phenology is likely temperature, controlled by GDD accumulation beginning immediately after snowmelt. In our transplant sites, slower phenological development on ridges is then a response to colder temperatures in the early growing season.

Leaf size was highly plastic, and responded by increasing when turfs were transplanted to snowbeds than when they were on ridges. Similarly, Walker et al. found larger leaf sizes produced under later snowmelt conditions in other alpine species [Walker et al. 1995] (but see [Stinson 2004]). The mechanisms driving larger leaf size in snowbeds are potentially higher temperatures, nutrient and/or water availabilities. Hudson et al. showed that

leaf size increased with warming in *Salix arctica*, another prostrate willow, and that this effect remained consistent through a long-term warming experiment [Hudson et al. 2011]. In our study area, later snowmelt timing resulted in warmer growing season temperatures, and nearby alpine sites showed both more bioavailable phosphorus and higher water availability soon after snowmelt [Little 2014], both factors which could potentially drive larger leaf sizes in snowbeds. At ridge microhabitats, a reduction in leaf size could have been driven by competition with taller alpine shrubs; however, a concurrent neighbor removal study examining interactions between *S. herbacea* and the surrounding vegetation community in the same research area showed that *S. herbacea* produced larger leaves on ridge microhabitats when growing in association with taller neighbours (Wheeler et al. unpublished). The rapid increase in leaf size under later snowmelt conditions means that shrubs can maximize photo-assimilation during the shorter growing seasons [Walker et al. 1995]. However, we could not detect an increase in clonal and sexual reproduction with bigger leaf size under later snowmelt conditions. In contrast, selection acted on leaf size with earlier snowmelt, since there was a decrease in flowering with smaller leaves on ridges; the fitness costs of these results are discussed below.

The proportion of flowering stems and stem number generally did not change in response to the destination microhabitat, but demonstrated strong effects of the origin microhabitat, despite corrections for initial stem number. Although these origin effects on flowering and stem numbers could indicate genetic differentiation, we must interpret them cautiously as there are several alternative explanations. The observed origin effects may be explained by an experimental period too short to detect differences in slow-responding *S. herbacea* traits (e.g. clonal growth), and might thus reflect maternal carry-over effects [Schwaegerle et al. 2000]. We accounted for potential carry-over effects due to differences in plant size by including the number of stems before as a covariate in the analyses. However, these effects might also have been influenced by a correlation with the age of the plant, which

is impossible to determine in the field. Another confounding factor could be that the transplanted soil might have contained soil biota and nutrients. However, transplanting with soil and soil biota might have also reduced the number of factors confounded with the shift in snowmelt timing, our main variable of interest. However, at minimum, we speculate that these slow responses in clonal and sexual reproduction might provide a buffer when snowmelt conditions change. Snowbed shrubs, with their high stem density, may prove particularly resistant, due to their potentially high flower production capacity and resource storage.

#### *No evidence for home site advantage*

Highly structured alpine landscapes with steep environmental gradients pose divergent selection pressures on, and lead to restricted gene flow between plant populations, which can promote local adaptation. A home-site advantage or other evidence for local adaptation has been found in many arctic and alpine plants [Kim and Donohue 2013, Byars et al. 2007, Gonzalo-Turpin and Hazard 2009, Bennington and Fetcher 2012], but see [Frei et al. 2013, Stanton and Galen 1997]. For our study species, ridge and snowbed microhabitats are often extensively temporally isolated by snowmelt timing [Wheeler et al. 2014, Cortés et al. 2014], which results in significant temporal separation in flowering times [Cortés et al. 2014].

Despite this phenological isolation between early and late snowbed sites, we did not find any indications for a home-site advantage to microhabitats, characterized by snowmelt timing, using either a sexual reproductive trait (proportion of flowering stems) and a clonal reproductive trait (the change in stem number) as fitness traits. This lack of a home-site advantage could have arisen through either carry-over effects or a lack of local adaptation, possibly due to high gene flow [Cortés et al. 2014], and particularly through the mechanism of high seed deposition in snowbed microhabitats, leading to little differentiation between ridge and snowbed plants [Cortés et al. 2014]. In a greenhouse experiment, where we used *S. herbacea* seeds and soil from the same study area, we found no indication for local adaptation

to soil biota in ridge and snowbed microhabitats [Sedlacek et al. 2014]. The lack of a home-site advantage suggests that there is no evidence for small-scale adaptive divergence within *S. herbacea* populations. This might be beneficial, if climate change leads to an advance in snowmelt timing and/or forces the dwarf shrub to migrate to new snowmelt microhabitats within the current range. However, a lack of small-scale adaptive divergence could also suggest limitations in the evolutionary potential of *S. herbacea*.

#### *Fitness consequences under an early snowmelt scenario*

In alpine regions climate change is predicted to drastically advance snowmelt timing and increase temperatures, together with changes in other abiotic and biotic factors, like soil moisture, nutrient availability and biotic interactions [Beniston et al. 2003]. These combined effects may strongly affect plant phenology, morphology and consequently fitness. In our study, we found no link between phenological development time and flowering probability, and thus no direct selection on phenological development time. However we were not able to disentangle potential positive and negative effects of a longer development time, which might have cancelled out each other. Under accelerated spring snowmelt conditions, a longer phenological development time might increase exposure to episodic freezing damage early in the season, when tissues are in the active growing stages and thus more vulnerable to damage. Since flower buds and flowers are especially vulnerable to freezing this might consequently lead to a reduction in sexual reproductive fitness [Inouye 2008, Ladinig et al. 2013]. Furthermore, Stinson showed negative selection on longer flowering development time, likely because of late-season declines of soil moisture in early snowmelt sites [Stinson 2004]. In contrast plant fitness might also benefit from a longer snow-free period, as there is more time for growth and resource allocation [Arft et al. 1999, Galen and Stanton 1991] though many dwarf shrubs show no link between advanced phenology and increased sexual reproduction [Wipf et al. 2009]. Finally, consequences of phenological development time on fitness may be

difficult to detect in a short time-frame, since spring freezing damage is episodic and may require repeated years of damage to deplete storage reserves [Wheeler et al. 2014, Molau 1997]. Further, in *S. herbacea*, flower buds are already preformed [Jones et al. 1997], and plants flower only once during the season; thus an advance of flowering would not extend the reproductive season or increase flower production in the short term.

Plant fitness is often demonstrated to benefit from relatively large leaves, by maximizing photosynthetic gains under cool, moist and shaded conditions [Givnish 1979]. In contrast, there might be a trade-off, selecting smaller leaves under hot, dry, high light and low nutrient conditions [Smith and Geller 1979]. Indeed, we found *S. herbacea* produced smaller leaves when they were on the drier, more exposed ridges than when they were in snowbeds. Nevertheless, we found positive selection on leaf size of plants on ridges (i.e. plants with bigger leaves were more likely to produce flowers). This suggests that the observed plastic reduction in leaf size under early snowmelt is actually not adaptive [van Kleunen and Fischer 2005]. Since, as previously discussed, competition does not appear to reduce leaf sizes on early snowmelt sites, the reduction in leaf size most likely reflects a passive plastic reduction as a consequence of lower resource availability on ridges. This passive reduction in leaf size might potentially lead to a reduction in flowering under early snowmelt conditions.

Under early snowmelt, the damage probability increased significantly. Although studies examining the frequency and severity of insect herbivore damage under earlier snowmelt are uncommon, increasing damage by herbivores and pathogens has been found in a long term warming experiment of alpine meadow plants by [Roy et al. 2004]. This trend is likely driven by a higher abundance of herbivores and pathogens and the prolonged exposure time of early snowmelt sites, which allow for greater developmental periods for growth and reproduction of the herbivore. Gerdol *et al.* demonstrated that leaf damage led to a decrease in plant fitness under earlier snowmelt conditions [Gerdol et al. 2013]. In this study, we found no evidence that higher damage resulted in a lower flowering probability or a decline in stem



numbers. However, a larger multi-year study showed reductions on female flowering probability of *S. herbacea* in the year following herbivory and fungal damage (Wheeler et al. unpublished). Thus, future studies should investigate male and female reproductive success in more detail, and assess changes in stem numbers over more years.

Despite responding to a change in snowmelt timing through plastic adjustment, plants might also be able to tolerate the new conditions through traits that increase their resistance. Our study suggests that plants from snowbeds can maintain a large size (stem numbers) in the short term (2 years) following a significant change in snowmelt timing. We suggest that this resistance is provided by increased resource storage due to high initial stem numbers. However, over the long term many alpine species have shown lagging population dynamics with currently occupied habitats becoming unsuitable, which leads to an extinction debt [Dullinger et al. 2012]. We speculate that many microhabitats may become unsuitable for *S. herbacea* due to earlier snowmelt conditions.

## **Conclusions**

In the studied populations of the alpine dwarf shrub *S. herbacea*, phenology and leaf size were strongly responsive to environmental changes triggered by shifts in snowmelt timing, but only changes in leaf size had a significant influence on plant fitness traits. Leaf damage probability was controlled by the environment, but appeared to have no fitness consequences in the short term. None of the *S. herbacea* individuals from early- or late snowmelt microhabitats demonstrated a home-site advantage, suggesting that the potential of *S. herbacea* to adapt to new snowmelt conditions might be limited. Sexual and clonal reproduction did not respond rapidly to snowmelt change, thus under early snowmelt conditions snowbed plants may still perform well in the short term, due to their high stem density relative to individuals from ridges. However, with accelerated spring snowmelt in the long term, exposure to damages and

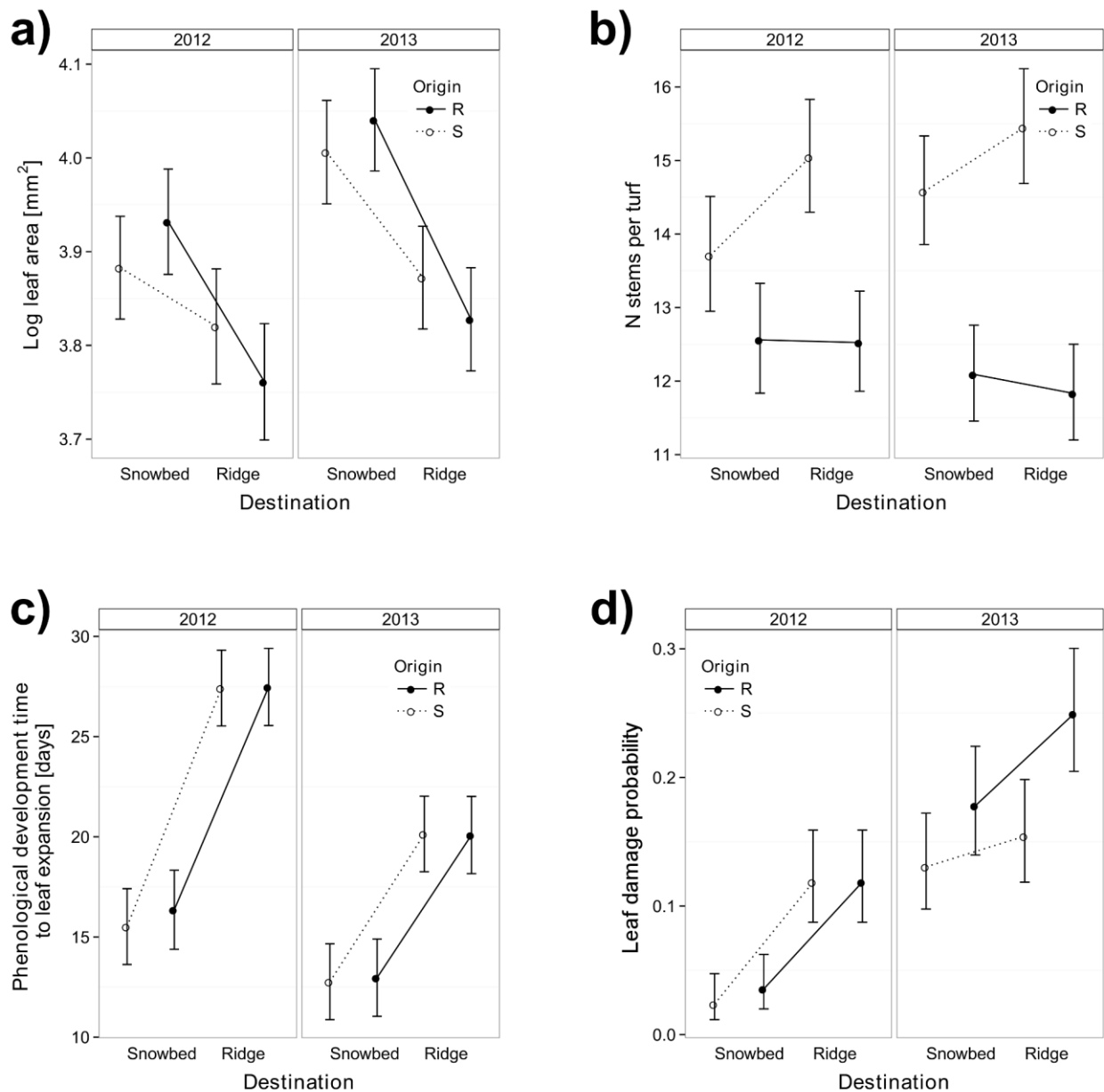
reductions in leaf size could lead to reduced flowering and consequently fitness reductions in the studied populations of *S. herbacea*.

### **Acknowledgements**

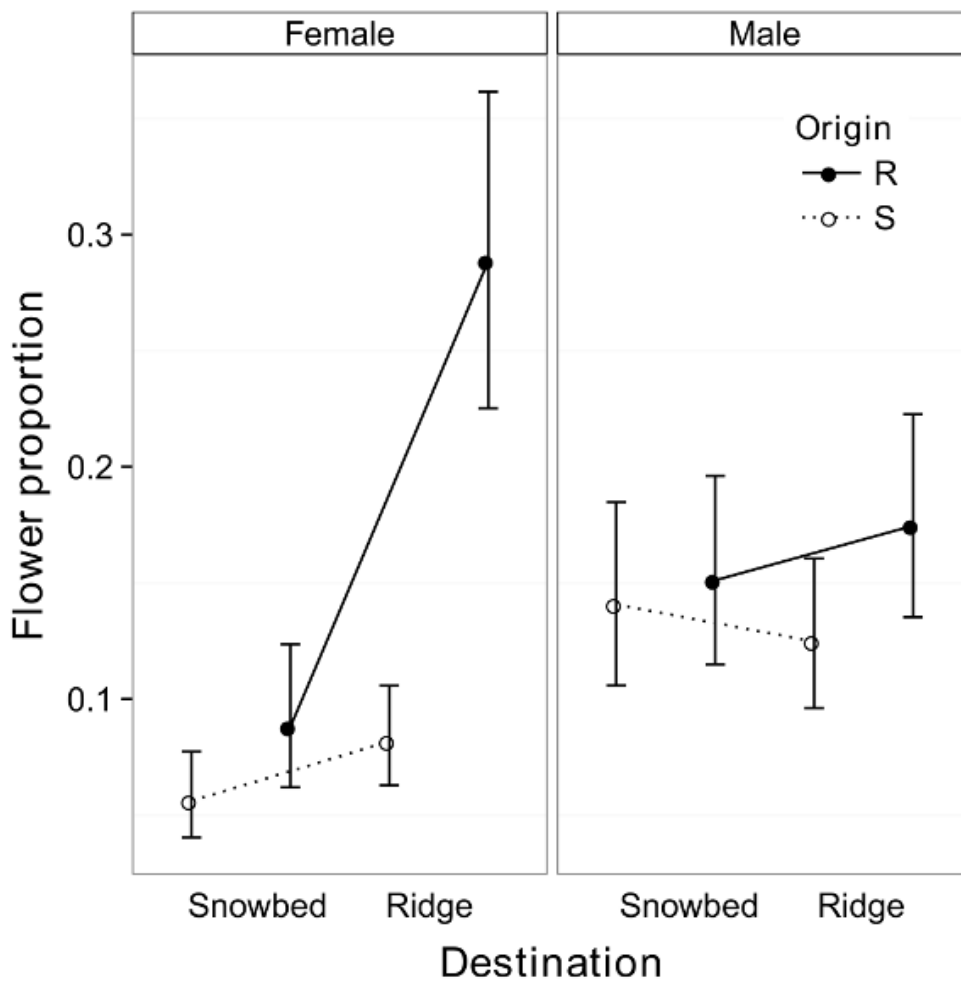
We thank three anonymous reviewers for constructive comments on an earlier version of the manuscript. We are grateful for the assistance of many dedicated field personnel in setting up and maintaining the transplant experiment, including C Little, G Klonner, S Häggberg, F Schnider, M Matteodo, M Liu, F Prahl, E Hallander, Y Bötsch, A Zieger, D Franciscus, A Nussbaumer, and C Scherrer.

**Table 1.** The effects of destination, origin, year and sex respectively, and their interactions, on the leaf size, stem number, phenology (duration from snowmelt to leaf expansion phenophase), leaf damage, ratio of flowering and ratio of fruiting stems of reciprocally transplanted *Salix herbacea* turfs. Ratio of flowering and fruiting stems was measured only in 2013, so year was excluded from these models. Ratio of initial stem number of 2011 was used as a covariate in the model for stem number (see Methods for details). Log-likelihood ratio tests were used to obtain  $\chi^2$  test statistic.

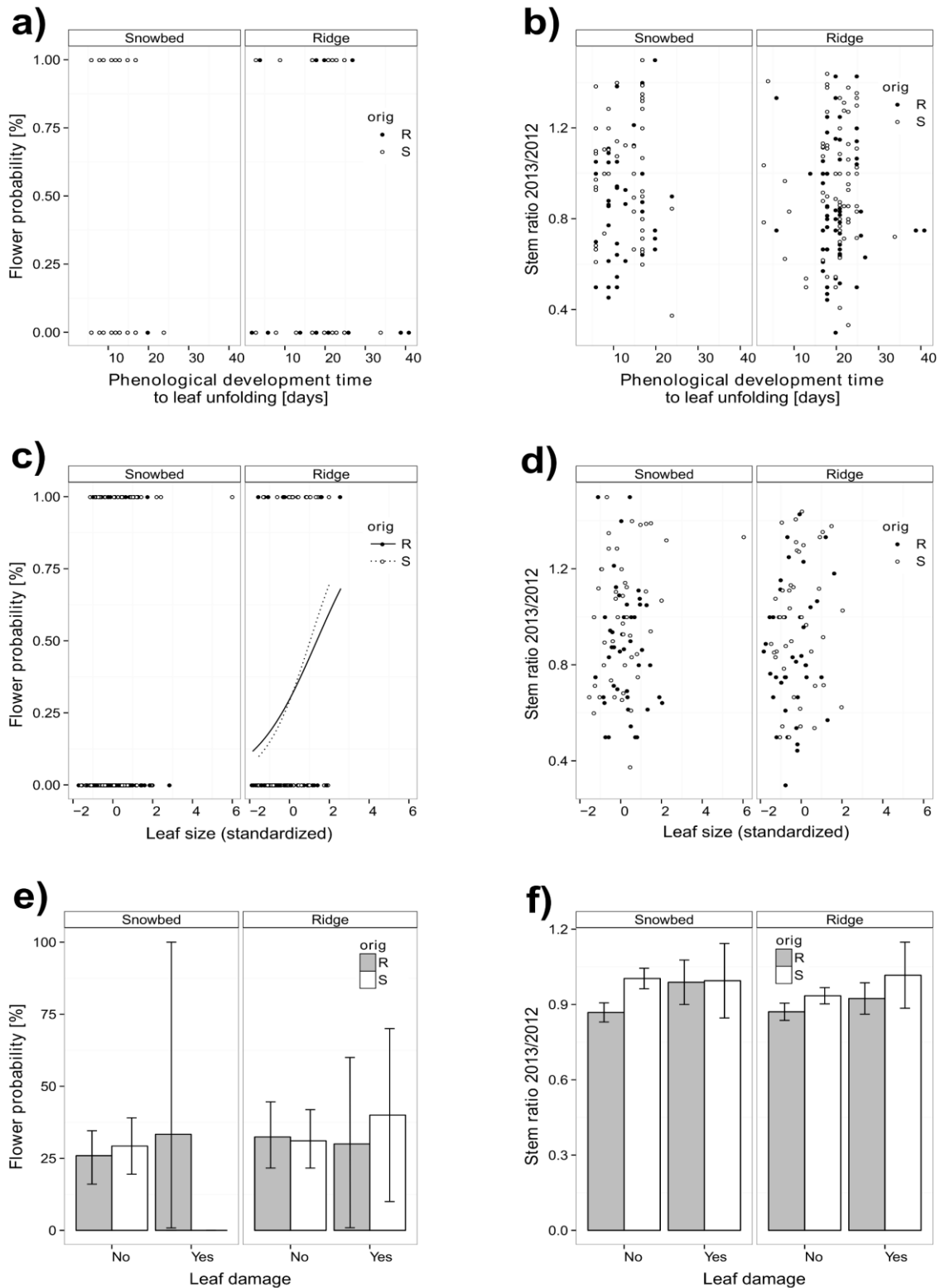
Source of Variation	Leaf size		Stem number		Onset of leaf expansion		Development time to leaf expansion		Leaf damage		Flowering stem ratio	
	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Stem number 2011	-	-	212.72	<0.001	-	-	-	-	-	-	-	-
Year	14.16	<0.001	0.09	0.767	41.30	<0.001	51.30	0.602	17.20	<0.001	-	-
Sex	-	-	-	-	-	-	-	-	-	-	1.29	0.257
Destination	4.70	0.030	0.40	0.526	25.33	<0.001	9.748	0.001	5.47	0.019	2.94	0.087
Origin	0.00	0.966	13.90	<0.001	2.13	0.144	0.168	0.681	2.37	0.123	6.35	0.012
Destination : Year	1.33	0.250	0.45	0.502	101.98	<0.001	9.33	0.002	4.27	0.039	-	-
Destination : Sex	-	-	-	-	-	-	-	-	-	-	3.52	0.061
Origin : Year	0.00	0.966	4.35	0.037	2.60	0.575	0.06	0.793	0.65	0.421	-	-
Origin : Sex	-	-	-	-	-	-	-	-	-	-	3.01	0.083
Origin : Destination	1.62	0.203	0.84	0.359	0.31	0.575	0.13	0.714	0.02	0.882	1.96	0.162
Origin : Destination : Year	0.09	0.763	0.04	0.852	0.39	0.531	0.03	0.849	0.30	0.584	-	-
Origin : Destination : Sex	-	-	-	-	-	-	-	-	-	-	0.62	0.433
<b>Random effects</b>	<b>Var</b>	<b>SD</b>	<b>Var</b>	<b>SD</b>	<b>Var</b>	<b>SD</b>	<b>Var</b>	<b>SD</b>	<b>Var</b>	<b>SD</b>	<b>Var</b>	<b>SD</b>
Turf/Patch/Plot/Site	0.004	0.066	0.243	1.557	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	2.709	0.520
Patch/Plot/Site	0.054	0.234	1.118	3.344	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.673	0.259
Plot/Site	0.008	0.089	0.008	0.029	0.188	4.342	16.012	4.001	0.109	1.046	<0.001	0.003
Site	<0.001	<0.001	<0.001	<0.001	1.808	1.344	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
Residual	0.078	0.280	-	-	25.000	5.000	50.221	7.087	-	-	-	-
<b>marginal <math>R^2</math>; conditional <math>R^2</math></b>	0.050	0.489	0.524	0.835	0.779	0.879	0.314	0.480	0.175	0.177	0.080	0.166



**Figure 1:** Leaf size (a), stem number (b), phenological development time (time from snowmelt to leaf expansion) (c), and leaf damage probability (d) of *Salix herbacea* turfs, reciprocally transplanted in 2011 between late exposed snowbed and early exposed ridge microhabitat sites in an alpine tundra site near Flüelapass, Switzerland. Turfs originating from ridges (R) are marked with solid lines and filled circles, turfs originating from snowbeds (S) with dashed lines and open circles. Error bars show standard errors.



**Figure 2:** Proportion of flowering female and male stems of *Salix herbacea* turfs, reciprocally transplanted in 2011 between late exposed snowbed and early exposed ridge microhabitat sites in an alpine tundra site near Flüelapass, Switzerland. Turfs originating from ridges (R) are marked with solid lines and filled circles, turfs originating from snowbeds (S) with dashed lines and open circles. Error bars show standard errors.



**Figure 3:** Selection gradients of *Salix herbacea* turfs growing in ridge and snowbed microhabitat sites. Turfs originating from ridges (R) are marked with solid lines and filled circles, turfs originating from snowbeds (S) with dashed lines and open circles. Lines are only shown when there was a significant ( $\alpha = 0.05$ ) selection coefficient. Errorbars show 95% CIs for flower probability (e) and standard errors for stem ratio (f).

## Appendix A. Supplemental Material

**Table A1.** Geographical coordinates, mean snowmelt date, mean growing season soil temperature and accumulated GDD over the growing season of the 6 ridge and 6 snowbed microhabitat sites used in a reciprocal transplant study established in 2011 in an alpine site near Flüelapass, Switzerland.

<b>Site</b>	<b>Latitude (°)</b>	<b>Longitude (°)</b>	<b>Snowmelt day 2012</b>	<b>Snowmelt day2013</b>	<b>Growing season soil Temp [°C] 2012</b>	<b>Growing season soil Temp [°C] 2013</b>	<b>GDD 2012</b>	<b>GDD 2013</b>
1-Ridge	46.74016667	9.96690556	135	163	8.5	11.5	1405	1226
2-Ridge	46.74026944	9.96581667	167	167	10.7	12.4	848	526
3-Ridge	46.74131389	9.96495000	136	164	11.2	12.3	1084	1189
4-Ridge	46.74095833	9.96466389	163	170	9.6	12.9	943	811
5-Ridge	46.74200000	9.96375833	154	164	10.4	9.8	1414	1330
6-Ridge	46.74340278	9.96161667	141	166	12.8	8.5	654	257
1-Snowbed	46.74008333	9.96637500	178	187	11.0	10.3	1234	1193
2-Snowbed	46.74068889	9.96563611	170	180	12.7	14.1	908	643
3-Snowbed	46.74110833	9.96565833	198	190	11.5	9.8	1117	715
4-Snowbed	46.74081111	9.96515000	198	195	12.8	13.4	1167	606
5-Snowbed	46.74218056	9.96396389	197	198	9.9	9.4	1074	1039
6-Snowbed	46.74323611	9.96196944	202	205	14.2	13.4	815	555

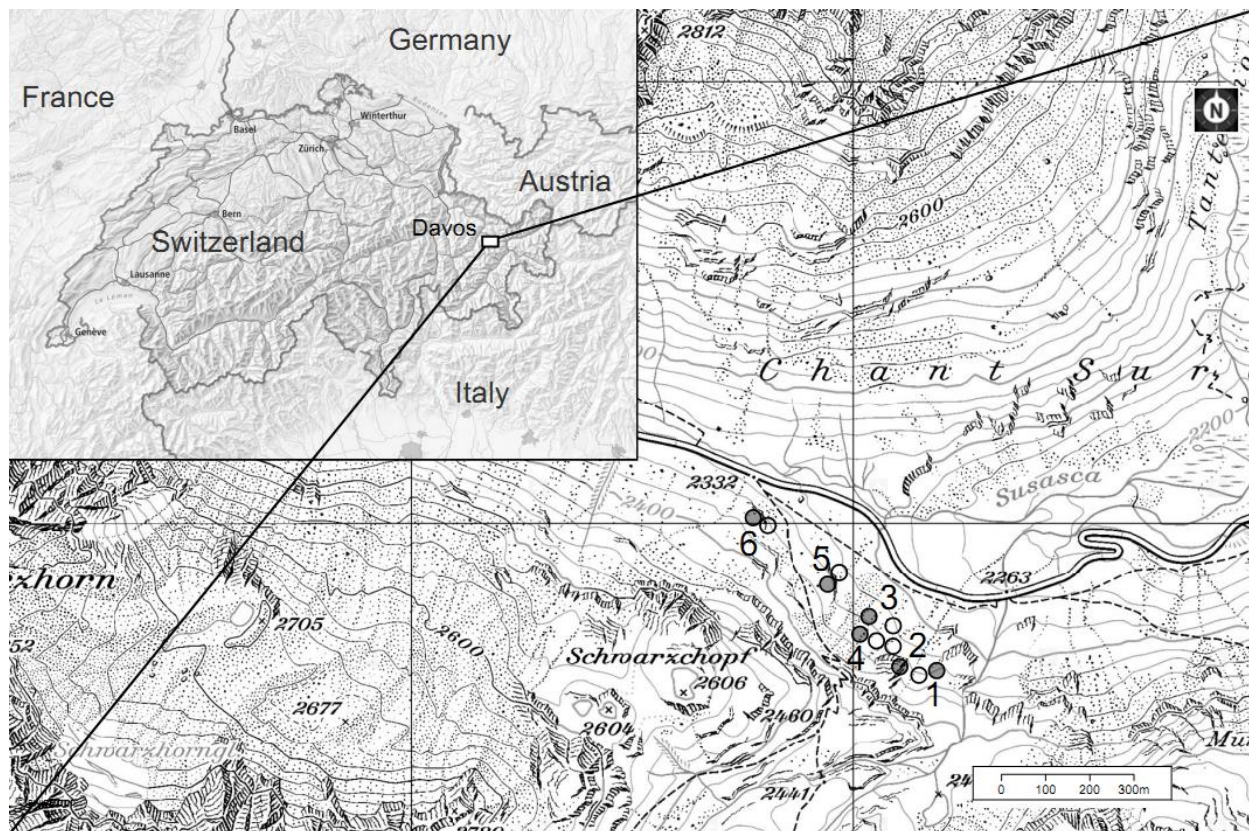
**Table A2.**

The effects of destination, origin, year and sex respectively, and their interactions, on phenology (day of year of flowering, duration from snowmelt to flowering, day of year of fruiting, duration from snowmelt to fruiting) of reciprocally transplanted *Salix herbacea* turfs. Log-likelihood ratio tests were used to obtain  $\chi^2$  test statistic.

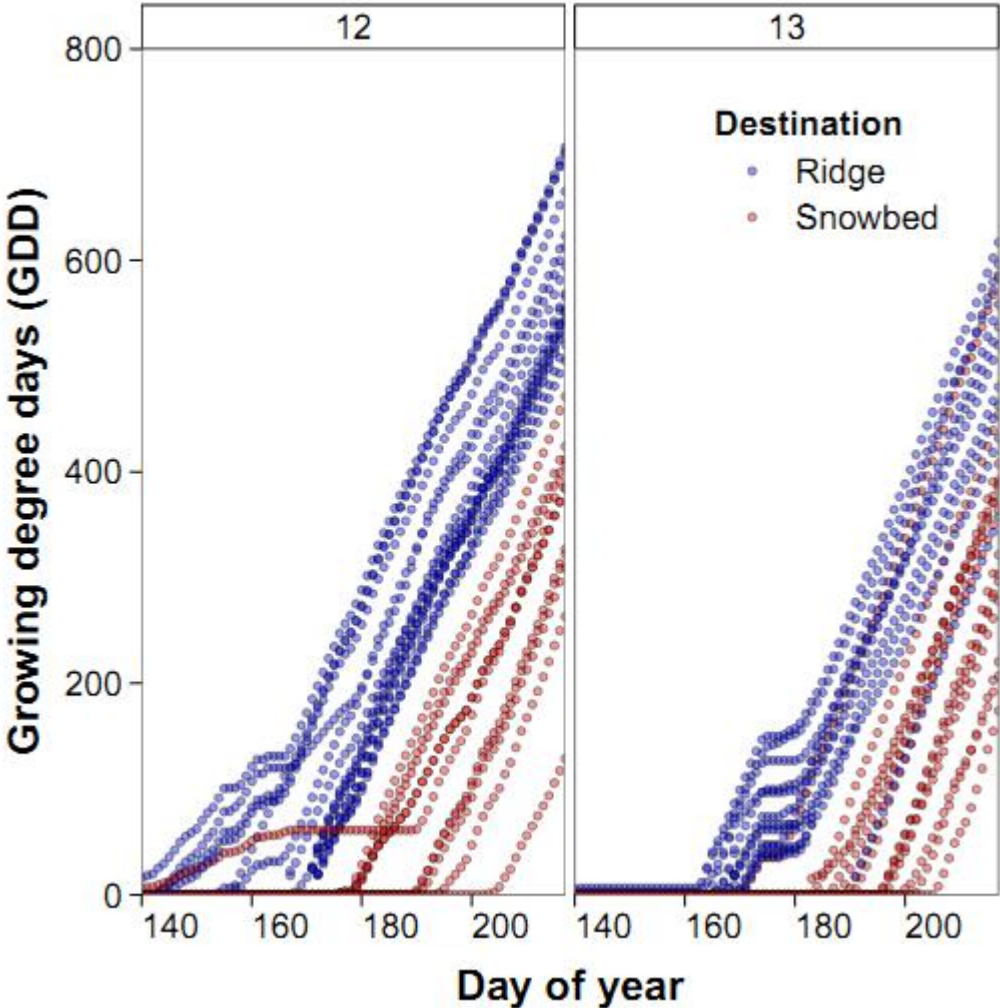
Source of Variation	Onset of leaf expansion		Onset of flowering		Development time to flowering		Onset of fruiting		Development time to fruiting	
	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Year	41.30	<0.001	59.09	<0.001	0.27	0.602	8.94	<0.002	7.284	0.006
Destination	25.33	<0.001	19.99	<0.001	9.53	0.002	23.35	<0.001	3.671	0.055
Origin	2.13	0.144	1.31	0.252	0.12	0.728	1.07	0.299	0.734	0.391
Destination : Year	101.98	<0.001	76.17	<0.001	0.53	0.468	13.79	<0.001	2.205	0.137
Origin : Year	2.60	0.106	0.01	0.917	1.51	0.219	0.24	0.624	0.002	0.96
Origin : Destination	0.31	0.575	<0.01	0.986	<0.01	0.984	0.21	0.641	0.165	0.683
Origin : Destination : Year	0.39	0.531	0.31	0.576	0.81	0.369	<0.01	0.937	0.975	0.754
Random effects	Var	SD	Var	SD	Var	SD	Var	SD	Var	SD
Turf/Patch/Plot/Site	<0.001	<0.001	7.019	2.649	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Patch/Plot/Site	<0.001	<0.001	7.151	2.674	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Plot/Site	18.85	4.342	42.85	6.546	8.864	2.977	21.42	4.628	74.4	8.626
Site	1.808	1.344	<0.001	<0.001	6.946	2.636	<0.001	<0.001	<0.001	<0.001
Residual	25	5	24.21	4.92	44.7	6.686	61.55	7.845	53.52	7.316
<b>marginal <math>R^2</math>; conditional <math>R^2</math></b>	0.779	0.879	0.743	0.923	0.343	0.613	0.717	0.79	0.247	0.685



**Figure A1.** Map of the study area near Davos (Switzerland), and locations of the six pairs of study sites (1-6), each consisting of one early exposed ridge microhabitat (filled circle) and one late exposed snowbed microhabitat (open circle).



**Figure A2.** Accumulated growing degree days on ridge- (blue) and snowbed sites (red) in 2012 and 2013. The data were collected on six ridge and six snowbed microhabitat sites used in a reciprocal transplant study established in 2011 in an alpine site near Flüelapass, Switzerland.



## Chapter 5

### Increased spring freezing vulnerability for alpine shrubs under early snowmelt

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*Salix herbacea* after budbreak during a spring snowfall, photo by Christian Rixen

## Abstract

Alpine dwarf shrub communities are phenologically linked with snowmelt timing, so early spring exposure may increase risk of freezing damage during early development, and consequently reduce seasonal growth. We examined whether environmental factors (duration of snowcover, elevation) influenced size, and the vulnerability of shrubs to spring freezing along elevational gradients and snow microhabitats by modelling the past frequency of spring freezing events.

We sampled biomass and measured size from *Salix herbacea*, *Vaccinium myrtillus*, *V. uliginosum* and *Loiseleuria procumbens* in late spring. Leaves were exposed to freezing temperatures to determine LT-50 (temperature at which 50% of specimens are killed) for each species and sampling site. By linking site snowmelt and temperatures to long-term climate measurements, we extrapolated the frequency of spring freezing events at each elevation, snow microhabitat and per species over 37 years.

Snowmelt timing was significantly driven by microhabitat effects, but independent of elevation. Shrub growth was neither enhanced nor reduced by earlier snowmelt, but decreased with elevation. Freezing resistance was strongly species-dependent, and did not differ along the elevation or snowmelt gradient. Microclimate extrapolation suggested that potentially lethal freezing events (May and June) occurred for three of the four species examined. Freezing events never occurred on late snowbeds, and increased in frequency with earlier snowmelt and higher elevation. Extrapolated freezing events showed a slight, non-significant increase over the 37-year record.

We suggest that earlier snowmelt does not enhance growth in four dominant alpine shrubs, but increases the risk of lethal spring freezing exposure for less freezing-resistant species.



## Introduction

Warming air temperatures due to climate change have been documented in many alpine systems, triggering reduced snowfall and earlier snowmelt (IPCC 2007, Pagter and Arora 2012, Rixen et al. 2012). Early snowmelt potentially represents a radical environmental change for many alpine plant communities, the phenology of which may be strongly linked with snowmelt timing in the spring (Keller and Körner 2003, Wipf and Rixen 2010). Dwarf shrubs represent a dominant vegetation type in the alpine zone above treeline, but despite increasing evidence for earlier snowmelt in alpine systems, the response of these communities to climate-induced changes in snowmelt timing has rarely been studied (Wipf and Rixen 2010, but see Wipf et al. 2009).

In alpine environments, a longer, warmer growing season might be associated with enhanced plant growth. This has been observed in Arctic shrub species, which have shown vegetative size and productivity increases with warming (e.g. Hudson et al. 2011, Elmendorf et al. 2012), indicating that vegetative size is a practical field measure for plant response to environmental change. However, accelerated alpine spring snowmelt is a critical ecosystem change: it can increase the length of photosynthetic period, increase drought exposure, alter timing of early-season phenology and, perhaps most importantly, increase exposure to spring freezing events (Inouye 2008). The few studies available examining alpine shrub response to accelerated snowmelt suggest growth is typically not enhanced under early snowmelt in temperate alpine habitats (Wipf et al. 2009, Rixen et al. 2010, Gerdol et al. 2013). Increased exposure to freezing events, as driven by earlier snowmelt timing, has also been shown to increase bud damage in alpine wildflowers (Inouye 2008), damage leaves and apical buds of alpine shrubs *Vaccinium* spp. and *Empetrum hermaphroditum* (Rixen et al. 2012), and reduce leaf

expansion, flowering and fruiting in *Vaccinium myrtillus* (Bokhorst et al. 2008, Gerdol et al. 2013).

Naturally-occurring spring and summer freezing events are episodic in alpine zones, and can reduce growth through the decrease of available metabolic energy, the reduction of water/nutrient uptake, and the damage/die-off of leaf tissue (Larcher 2003, Rixen et al. 2012). Spring freezing rarely kills alpine plants, but these cold events have been shown to significantly reduce both above-ground biomass and reproductive output, and can potentially reduce the abundance and competitive ability of the species at the population level (Molau 1997, Bokhorst et al. 2008). Some alpine plants may delay spring phenology until the risk of freezing damage to sensitive new tissue has passed (Körner 2003), but since this strategy is most likely controlled by photoperiodism, it probably does not occur in alpine sites, as daylength at time of snowmelt is already near its annual maximum (a 15 h day-length threshold has been suggested for the onset of alpine plant growth, Heide 2001). Freezing resistance differs strongly between species and functional groups, and has been extensively studied previously, with many studies also including alpine shrubs (e.g. Sakai and Larcher 1987, Taschler and Neuner 2004, Martin et al. 2010). However, while studies have examined freezing resistance along elevational gradients (Taschler and Neuner 2004, Sierra-Almeida et al. 2009), soil warming gradients (Martin et al. 2010), and between snow microhabitats (Bannister et al. 2005), to our knowledge no one has examined the same species along elevation and snowmelt gradients within a single study. Wipf et al. (2009) examined the impacts of accelerated snowmelt on growth and phenology in alpine shrubs and speculated about the role of spring frosts in alpine shrub failure to respond positively to longer seasons, but did not quantify shrub freezing resistance, or examine the long-term frequency of spring freezing events or community response along the elevational gradient. In complex alpine

ecosystems, it is critical to understand the interplay between ongoing changes in elevational temperature gradients, snowmelt effects as controlled by microtopography, and species-specific freezing resistance responses in order to predict the effects of earlier spring snowmelt on alpine shrubs.

In this study, we investigated the impact of growing season length (triggered by natural snowmelt timing) and elevation on shrub growth and risk of exposure to potentially lethal spring and early summer freezing events in four alpine dwarf shrubs. We hypothesized that earlier snowmelt does not enhance shrub performance because earlier snowmelt increases the risk of exposure to damaging spring freezing events, thus failing to enhance or even reducing seasonal growth. We sampled shrub biomass with mature leaf tissue along an elevational and snowmelt gradient and exposed it to simulated freezing conditions in order to: i) assess environmental factors (duration of snow cover, elevation) influencing functional growth traits (leaf area, annual shoot increment, stem length); ii) compare interspecific and intraspecific freezing resistance (measured as LT-50, temperature at which 50% of specimens are killed) along the elevational and snowmelt gradients and iii) determine the potential vulnerability of mature leaf tissue in shrubs to post-snowmelt freezing along the elevational and snowmelt gradients through the frequency of species-specific spring lethal freezing events by using long-term meteorological data. The results of this experiment will add to our understanding of alpine shrub responses to accelerated snowmelt, and aid in predicting potential changes in the shrub community driven by climate warming.

## Methods

### *Study site and sampling design*

Our study transect is located on the northeast-exposed face of the Jakobshorn peak (2590 m asl), above the Stillberg Long-Term Afforestation Research Area (9°52'E, 46°46'N) in the central Alps, Switzerland. The transect covers an elevational gradient of 2100 to 2500 m asl, from just above the natural climatic treeline to the peak. The climate record at 2100 m asl (1975 to present) shows annual mean precipitation at 1150 mm, with 48% falling between June-September. Snowfall can occur in all months, with continuous snow cover occurring usually from mid-October to late May (Wipf et al. 2009). Mean spring air temperatures (May=4.5°C, June=7.2°C) gradually warm to summer mean temperature peaks (July=10.0°C, August=9.8°C), with only episodic freezing events between May and September. The 37-year climatic record at this site indicates that mean June-July-August air temperatures have warmed significantly, and that snow is melting significantly earlier in the spring, at a rate of 3.5 days per decade (Rixen et al. 2012).

Sites were established at four elevations along the 400m gradient, at an early-exposure ridge and a late snowbed microhabitat, for a total of eight sites (Table 1). Relatively early-melting snowbed sites were paired with very early-exposure ridges in order to ensure that sampled shrubs would have developed to similar leaf phenophases. In-situ soil temperature loggers (iButton, Maxim Integrated, San Jose USA) recorded 2012 soil temperatures (2 hour intervals) at each site at 5 cm soil depth and were used in conjunction with field observation to determine day of snowmelt (date in spring when soil temperature rose abruptly from the near-0°C characteristic of snow cover). Growing degree days (GDD; sum of daily mean temperatures above 5°C) were calculated from snowmelt to the end of August for each site. Total season length for each site was calculated from snowmelt day to first day in autumn when mean air temperatures fell below 0°C.



Four common and dominant alpine dwarf shrub species (2-3 specimens per site) were sampled at each site for this study: evergreen *Loiseleuria procumbens* (L.) Desv., Family Ericaceae, and deciduous shrubs *Vaccinium myrtillus* L., Family Ericaceae, *Vaccinium uliginosum* L. ssp. *gaultherioides*, Family Ericaceae and *Salix herbacea* L., Family Salicaceae. Shrub stem and leaf biomass were sampled during a one-day period on 23-June-2012 at similar phenological stages (mature, newly opened leaves) to minimize potential intraspecies differences in LT-50. The one-day-sampling can only be considered a snapshot in LT-50 because cold hardiness continuously changes through plant life stages (Sakai and Larcher 1987). However, our sampling period was ideal for our purposes because leaves were already fully developed but without signs of damage like herbivory or senescence yet; such conditions were necessary to cover our research design including the gradients in elevation and snowmelt. Furthermore, air temperatures in the 7 days prior to sampling (Table 1) were never below 0°C, making it unlikely that the sampled shrubs were unusually cold-hardened. Functional growth traits (mean leaf area calculated from leaf length x leaf width, mean stem length) were measured and used as the primary growth variables.

#### *Freezing resistance (LT-50) determination*

Live shrub biomass (stems and leaves) was immediately placed in cooling boxes after sampling and transported to the freezing lab facility at the University of Basel (Basel, Switzerland) within a day. Specimen biomass samples (6-8 branches with intact, healthy leaves) were separated into nine subsamples, wrapped in paper and aluminum foil to buffer temperature fluctuations (effective to 0.1K; Larcher et al. 2010) and to prevent water loss and mechanical damage. Each subsample was exposed to minimum temperature treatments (4°C, -2°C, -4°C , -

7°C, -11°C, -14 °C, -16°C, -19°C, -20°C) in a computer-controlled freezing system, where temperature was started at 4°C, decreased to the minimum temperature at 3 K per hour, maintained for 4 hours, and then returned to 4°C at 3 K per hour. Damage to leaf tissue was visually quantified on a 10% scale (0 to 100% damage) to determine the lethal kill temperature for mature leaf tissue in each specimen, with damage to greater than 70% of the leaves classified as a 'kill'. Visual quantification of damage (surface darkening and limpness from loss of turgor pressure) for these species is strongly correlated with leaf tissue electrolyte leakage associated with freezing damage (Martin et al. 2010). LT-50 for each specimen was determined by fitting a sigmoid curve to the eight points on the temperature-percent damage plot and calculating the inflection point (Martin et al. 2010). If the sigmoid curve lacked a defined inflection point, the LT-50 for that specimen was not included in the analysis; however, this was relatively infrequent (less than 10% of the data points). Data were pooled by species for each site, and LT-50 was then calculated for each species at each sampling site.

### *Spring lethal cold events*

Lethal spring freezing events were defined as spring (May and June) daily air temperature minima either at or below the mean LT-50 for a shrub species occurring a minimum of 5 days after projected site snowmelt at 40 microsites (20 ridges, 20 snowbeds) along the elevational gradient (sites separated by a mean elevational distance of approximately 50 m). The minimum five day threshold after projected snowmelt date was implemented in order to maximize the probability of shrubs having undergone budbreak prior to a freezing event occurring. The in-situ climate station at 2100 m asl recorded hourly air temperature (2 m) and annual date of snowmelt from 1975 to 2012. Snowmelt dates measured at each of the 20 sites in 2012 were used to relate snowmelt date at a given site to the date recorded at the climate station. Snowmelt dates in 2012

were representative of the long-term mean in the region (1 day later than the 37-year mean). Nearby snow depth accumulations have been shown to be similar from year to year (Schirmer et al. 2011), and the order of site emergence from snow has been relatively consistent over two years (2011-2012) of monitoring (Wheeler, unpublished data). This relative snowmelt date for each site was then used to estimate date of snowmelt for all transect sites between 1975 and 2012. Site air temperatures after predicted snowmelt were extrapolated for each site using a lapse rate of  $0.52 \text{ K } 100\text{m}^{-1}$  (averaged from long-term May-June data at  $n=15$  climate stations in Swiss Alps; Kollas et al. 2012). This lapse rate value is generally similar to those calculated for long-term daily temperature minima under a range of alpine atmospheric conditions in spring months (Kirchner et al. 2013). Frequency and yearly recurrence of lethal spring freezing events were then calculated for each species at each site for the past 37 years in order to extrapolate relative spring freezing event frequency along the elevational and snowmelt gradients, and over multiple decades. Since snowmelt day for each site over the long-term record is extrapolated from the 2012 record and not determined from site observation, this is meant as an indicator of relative changes of freezing frequency along the gradients, and not a direct prediction of the actual (absolute) freezing frequency.

### *Statistical analyses*

To quantify environmental differences along the elevational gradient and between snow microhabitats, we used general linear models to analyze both snowmelt timing and soil temperatures (response variables) along the elevational gradient and between microhabitat types (explanatory variables). Growing season soil temperature was significantly negatively correlated with elevation and with earlier snowmelt day, and demonstrated similar trends as the results for elevation in the individual linear regressions; as elevational trends were slightly stronger,

elevation was chosen as the explanatory variable and soil temperature was dropped from the analysis. We used general linear models (Type 3 error) to analyze growth traits (mean leaf area, mean stem length) for each species individually, with elevation and snowmelt day as explanatory variables. We analyzed freezing resistance (LT-50) also with general linear models but with species, elevation and snowmelt day as explanatory variables. Because species was highly significant, we also analyzed freezing resistance on a species basis similar to the growth traits. Mean leaf area and mean stem length were log-transformed to ensure normality. Assumptions of linearity and homoscedasticity were confirmed using standard diagnostic plots. We used generalized linear models (quasipoisson distribution) to analyse the frequency of spring freezing events for each species over the 37-year record, with centered elevation and centered snowmelt day as explanatory variables. However, results are only shown for *V. myrtillus*, since it was the only species exposed to relatively frequent spring freezing events all along the elevational gradient. All analyses were carried out in R v.2.15.1.

## Results

### *Snowmelt and elevation*

Snowmelt timing (Table 1) was typical for the long-term average in 2012, occurring 1 day later than the long-term mean (data not shown). Snowmelt occurred significantly earlier on ridges (N=4) relative to snowbed microhabitats (N=4; mean ridge snowmelt day of year (DOY)= 142, mean snowbed snowmelt DOY= 159,  $F=20.1$ ,  $p<0.0001$ ); however, elevation had no significant effect on snowmelt timing ( $F=0.24$ ,  $p=0.62$ ); this indicates that local microhabitat effects are the primary driver of snowmelt timing. These strong local effects may be characteristic at the

landscape scale, as two similar transects on other mountains nearby also failed to demonstrate consistent elevational-snowmelt effects over multiple years (data not shown). We assumed that growing season ended on September 14 for all sites, when daily mean air temperature fell to  $-1.36^{\circ}\text{C}$  at 2090m asl. Hence, the mean ridge growing season length was 116 days, while mean snowbed growing season length was 99 days. The 17-day difference between ridge and snowbed meltout timing represents about 17% of the mean growing season length at snowbed sites.

Ridge and snowbed microhabitats significantly differed in soil temperature after snowmelt in June, but these differences disappeared over the course of the growing season; June GDD significantly increased with earlier snowmelt day (mean ridge June GDD=278, mean snowbed June GDD=214;  $F=17.8$ ,  $p=0.0002$ ), but this difference between microhabitat types was not present over the course of the entire summer, as growing season GDD (snowmelt to the end of August) was not significantly different (mean ridge GDD=1202, mean snowbed GDD=1077;  $F=1.2$ ,  $p=0.26$ ). Mean June temperatures were not significantly different (mean ridge June  $=10.0^{\circ}\text{C}$ , mean snowbed June  $=9.85^{\circ}\text{C}$ ;  $F=1.7$ ,  $p=0.20$ ), while mean soil temperatures over the entire growing season were significantly higher at later snowmelt sites (mean ridge  $=10.0^{\circ}\text{C}$ , mean snowbed  $=10.4^{\circ}\text{C}$ ;  $F=13.2$ ,  $p=0.001$ ).

June GDD and growing season GDD decreased with marginal significance along the elevational gradient ( $t=-1.96$ ,  $r=-0.34$ ,  $p=0.059$  for June GDD;  $t=-1.84$ ,  $r=-0.32$ ,  $p=0.075$  for season GDD) while mean June and mean growing season temperature both decreased significantly elevationally ( $t=-5.17$ ,  $r=-0.69$ ,  $p<0.0001$  for mean June temperature;  $t=-2.49$ ,  $r=-0.41$ ,  $p=0.018$  for mean season temperature).

### *Growth performance along environmental gradients*

The prostrate shrubs *S. herbacea* and *L. procumbens* showed no elevational growth response (Fig. 1, Fig. 2). However, the higher-statured *V. myrtillus* and *V. uliginosum* both demonstrated a significant negative growth response to elevation, with a significant decrease in mature leaf area with increasing elevation (Figure 1; DF=1, F= 22.42, p=0.0032; DF= 1 F=12.74, p=.012). *V. uliginosum*, the tallest shrub in this study, further demonstrated a significant decrease in mean stem length with elevation (Figure 2; DF=1, F= 14.82, p=0.0085). No shrub species demonstrated a significant growth response to snowmelt timing. This suggests that differences in snowmelt and growing season length in the observed range have a much weaker effect on shrub growth performance than other environmental drivers (e.g. elevation).

#### *Vulnerability to spring freezing damage*

Pooled spring freezing resistance (LT-50) of leaves was significantly different between species (DF=3, F=22.34, p<0.0001), with low-stature evergreen *L. procumbens* having the highest freezing resistance (N=8, -11.0°C), followed by *V. uliginosum* (N=8, -9.0°C), *S. herbacea* (N=8, -7.0°C) and *V. myrtillus* (N=8, -5.1°C). Spring freezing resistance was not found to be significantly different along the elevational and snowmelt gradients in any shrub species (elevation gradient *L. procumbens* DF=1, F=1.11, p=0.33; *S. herbacea* DF=1, F=0.92, p= 0.38; *V. uliginosum* DF=1, F=0.17, p=0.70; *V. myrtillus* DF=1, F=1.62, p=0.25; snowmelt gradient *L. procumbens* DF=1, F=0.066, p=0.81; *S. herbacea* DF=1, F=1.31, p=0.30; *V. uliginosum* DF=1, F=0.0024, p=0.96; *V. myrtillus* DF=1, F=1.21, p=0.32). This may indicate species-specific (i.e. genotypic) freezing resistance, as opposed to an environmentally driven effect (i.e. phenotypic), specifically in mature leaf tissue.

Based on our extrapolations, no lethal cold events occurred in July between 1975 and 2012 for any species, indicating leaf-damaging cold events are primarily a spring (May-June)

phenomenon. Figure 4 demonstrates spring (May-June) temperature minima over 37 years, which we used to extrapolate the frequency of lethal spring cold events after snowmelt occurring at or below species LT-50 thresholds. Lethal spring events did not occur on snowbeds and thus lethal event frequency analyses were restricted to ridge microhabitats. No lethal spring cold events were documented on the exposed ridges for *L. procumbens* in the 37-year temperature record. Spring lethal cold events for *V. uliginosum* were extremely rare and were only detected in 2012 (the last year on record). Lethal cold events for *S. herbacea* occurred at a significantly lower frequency than for *V. myrtillus* on the 20 exposed ridges over the 37-year temperature record (*V. myrtillus* total N events=375, *S. herbacea* total N events = 75, DF=1, F=40.08,  $p<0.0001$ ). *S. herbacea* lethal freezing events were also generally restricted to the highest elevational sites, while *V. myrtillus* experienced lethal freezing at all elevations. Because damaging spring freezing events were relatively abundant for *V. myrtillus* only, we restricted the statistical analyses of temporal and spatial occurrences of freezing events to this species.

#### *Lethal spring cold events in V. myrtillus on the spatial and temporal scale*

The frequency of lethal spring cold events on exposed ridges was stable over the 37-year record, and even demonstrated a slight increase, although the increase was not significant (Figure 4a;  $t=-0.60$ ,  $p=0.55$ ). Thus, considering the significant increase in local air temperature over the same period of time ( $0.58^{\circ}\text{C}$  summer warming per decade since 1975; Rixen et al. 2012), our study counter-intuitively suggests that warming spring air temperatures are not reducing the number of damaging spring freezing events. When lethal spring cold events are pooled across all years, *V. myrtillus* is at significantly increased risk of exposure with both increasing elevation (Figure 4b;  $t=6.89$ ,  $p<0.0001$ ) and earlier snowmelt (Figure 4c;  $t=-7.16$ ,  $p<0.0001$ ), coupled with

significant interactive effects ( $t=3.58$ ,  $p=0.00038$ ), with high-elevation early-exposed ridge habitats being the most vulnerable to freezing exposure.

Supplemental Table 1 lists all model variables, F, t and p values (Online Resource 1).

## Discussion

In our alpine system, localized microhabitat effects were the primary drivers of snowmelt. Alpine dwarf shrub growth was not enhanced by natural early snowmelt, and different species at leaf maturity demonstrated significant differences in vulnerability to spring freezing exposure along the snowmelt and elevational gradient. Because freezing resistance at leaf maturity within a species did not change with elevation or microhabitat, both higher elevation and earlier snowmelt microhabitats had a significantly higher risk of spring freezing exposure. Spring freezing events increased slightly, although not significantly, over 37 years despite local warming spring air temperatures.

### *Growth performance along environmental gradients*

Non-prostrate shrubs (*V. myrtillus* and *V. uliginosum*) demonstrated a significant elevational growth response in this study. This represents either a true growth response or different resource allocation patterns along the environmental gradient (Sakai et al. 2003). True growth including below-ground biomass production can often not be accurately determined in clonal alpine shrubs (Körner 2003). However, numerous studies have shown that vegetative above-ground structure size, such as shrub height, leaf size or stem length, is a practical variable for plant and community levels responses (Hudson et al. 2011, Elmendorf et al. 2012). In our set



of species, it is likely that shrub stature plays a major role in this observed growth response to elevation. Taller shrubs, like *V. myrtillus* and *V. uliginosum*, are more closely coupled to elevationally-lapsed atmospheric temperatures (similar to arborescent species), whereas prostrate shrubs may be largely decoupled from free atmospheric conditions, and are likely more strongly exposed to and controlled by microclimatic conditions (Körner 2012). Consequently, fluctuations in air temperature are also more pronounced for taller shrubs, as prostrate species likely profit from delayed night heat loss due to the heat retention capacity of the soil and litter layers (Sakai and Larcher 1987, Körner 2003).

We found no evidence of enhanced shrub growth as a response to earlier snowmelt timing and thus a longer growing season, and there is increasing evidence in the literature that suggests a longer growing season does not necessarily benefit alpine plant growth. Although Mallik et al. (2011) demonstrated reduced growth in Arctic shrub *Cassiope tetragona* under delayed snowmelt conditions, and *S. herbacea* has demonstrated natural increased growth under longer growing seasons (Wijk 1986), a review by Wipf and Rixen (2010) examining growth responses in snow manipulation experiments indicated no clear plant growth response pattern to delayed snowmelt. They suggested instead that responses depended on functional group, studied microhabitat type, and the magnitude of snowmelt timing change. The same review concluded that alpine plant growth responses to earlier snowmelt have been understudied (Wipf and Rixen 2010). Conifer tree species at and above treeline do not demonstrate strong growth responses to snowmelt timing (Barbeito et al. 2012), and in the few experimentally-accelerated snowmelt studies, very few species demonstrated enhanced growth, with only *Empetrum nigrum* and *L. procumbens*, two species characteristic to snow-poor microsites, showing increased stem elongation in response to earlier snowmelt (Wipf et al. 2009, Wipf 2010). Gerdol et al. (2013) even demonstrated size reductions in *V. myrtillus* under advanced snowmelt conditions. This prevailing lack of enhanced

shrub growth, in both this and other studies, may be explained by the nature of temperature accumulation on ridge and snowbed microhabitat sites. Ridge sites emerge from snow earlier, and thus begin accumulating temperature earlier, but have lower season-long temperature means than late snowmelt microsites. As such, shrub communities growing on early snow-free sites have longer growing seasons with greater early-season temperature sums, but colder mean temperatures and greater likelihood of exposure to spring cold events (Inouye 2008, Wipf et al. 2009, Gerdol et al. 2013). These spring cold events, occurring during active growth phases (i.e. development and maturation of leaf tissue) have the potential to severely damage plant biomass and can potentially limit new growth (Wipf et al. 2009, Sierra-Almeida and Cavieres 2012).

#### *Spring freezing resistance*

Freezing resistance was found to be species-specific and consistent with the existing literature for the shrub species examined (Taschler and Neuner 2004, Martin et al. 2010), although LT-50 dependence on phenophase makes comparisons across studies difficult (Sakai and Larcher 1987, Lenz et al. 2013). Measured freezing resistance also corresponded roughly to ecological niche for each species, with prostrate, ridge specialist evergreen *L. procumbens* having a higher freezing resistance than prostrate deciduous snowbed specialist *S. herbacea* or taller-growing, ubiquitous *V. myrtillus*. This corresponds with Bannister et al. (2005), who linked freezing resistance to microhabitat type, with snowbed and sheltered species having poorer frost resistance than early-exposed ridge species. *V. uliginosum* fell outside this trend, but its higher freezing resistance compared to *V. myrtillus* was consistent with the literature (Taschler and Neuner 2004, Martin et al. 2010). Prostrate shrubs did not necessarily have greater freezing resistance than taller species in our study, corresponding to previous findings that found no

correlation between species height and freezing resistance in temperate alpine and Ecuadorian páramo species (Taschler and Neuner 2004, Sklenar et al. 2010).

At time of sampling, freezing resistance was likely at a low point for each species in the spring period, as mature xylem and adult leaves have a lower freezing resistance than post-winter buds (Sakai and Larcher 1987). In the current study, we only sampled newly mature leaves along the entire gradient in order to minimize bias on LT-50 by different leaf-developmental stages between sites. Thus, we are drawing conclusions about the freezing vulnerability of mature leaves. Freezing resistance likely changed strongly earlier in the growing season, but after leaf maturity, freezing resistance will to change to a much lesser extent, unless in response to an unusual freezing event; Venn et al. (2013) and Ladinig et al. (2013) demonstrated that foliar tissue and vegetative shoots generally maintain a relatively consistent frost resistance through the growing season. We did not find any significant differences in LT-50 of mature leaves between snow microhabitats or along the elevational gradient. Similarly, Bannister et al. (2005) also found no consistent difference in freezing resistance in alpine species exposed early in spring compared to the same species growing in later snowbeds. While Taschler and Neuner (2004) found that, in species with similar growth forms, the ones with higher distribution limits had higher freezing resistance, others reported no strong or consistent evidence for elevational decrease in LT-50 in mature leaves of Swiss broadleaf trees, grasses, or other alpine species (Márquez et al. 2007, Sierra-Almeida et al. 2009, Lenz unpublished data). This suggests that shrub freezing resistance at leaf maturity is not closely tied to environmental conditions. In temperate broadleaf trees, it has been suggested that some species delay flushing and development until late enough that freezing events are highly unlikely (Lenz et al. 2013). This is less likely to be the case in alpine shrubs, however, since bud-break in these species is closely linked to snowmelt timing and not controlled by photoperiod or air temperatures before flushing (Sakai and Larcher 1987, Rixen et al. 2012).

Cold hardening may act as mechanism for increasing plant resistance to freezing. Extreme cold hardening has been shown to occur in grass species primed at subzero temperatures for four or more days (Bykova and Sage 2012). This hardening, however, occurred under winter conditions, which may not accurately reflect the episodic nature of spring freezing events, which are unlikely to be preceded by a long period of subzero temperatures (Rixen et al. 2012). In spring, when alpine shrub *R. ferrugineum* was exposed to in-situ freezing under field conditions at treeline, some cold hardening occurred after three days. However, hardening in this shrub was demonstrated to be slowed by a combination of warm daytime temperatures (+19°C) followed by night freezing (Neuner et al. 1999). This multi-day lag period prior to cold hardening, exacerbated by strong diurnal temperature differences and coupled with the episodic nature of spring freezing, suggests that shrubs in this study were not cold-hardened at the time of sampling. It also suggests that in the absence of prolonged cold spring temperatures, spring cold hardening is unlikely to function as a mechanism of mature leaf tissue freezing resistance in alpine shrubs. Indeed, Venn et al. (2013) found no consistent evidence for cold hardening in leaf tissue of alpine plants during the growing season. In the current study, we did not find differences in freezing resistance along the elevational gradient or among microhabitats within a species, suggesting only a limited potential to change LT50 in mature leaves in late spring, in the absence of a prolonged freezing event.

The similarity in mature leaf freezing resistance for each species along the snowmelt and elevational gradient greatly simplifies assumptions about the shrub community's vulnerability to spring freezing events. Since mature leaf tissue LT-50 was linked to species, and not microsite conditions, it can be assumed that, after emergence from snow cover, mature leaves of a given species are more or less equally vulnerable to spring freezing across the local species distribution range. Our results suggest that the upper distribution limits for these alpine shrub species may be

at least partially determined by spring cold temperature tolerances. Low-statured tree seedlings are known to establish above their elevational limit in microhabitats that regulate favourable growing temperatures (Sundqvist et al. 2008, Wheeler et al. 2011), and lower-elevation shrubs have been shown to track summer and winter warming into higher elevation snowbed sites in Sweden (Kullman 2002), suggesting colder temperatures limit upper distributions. Since episodic cold events are common after snowmelt (Körner 2003) and tend to strike shrubs during vulnerable active growth stages (Sierra-Almeida and Cavieres 2009), these are possibly one of the limiting factors that reduce shrub performance (Wipf et al. 2009) and could thus control higher shrub distribution.

#### *Risk of spring freezing events*

*V. myrtillus* faces a far greater risk of spring freezing than the rest of the dominant shrub community. This is determined by both its poor freezing resistance and ubiquitous presence in early-exposure microsites. Since resistance to freezing after dehardening is considered a strong filter for alpine plant distribution (Körner 2003), this suggests that *V. myrtillus* opportunistically responds to the episodic nature of spring freezing in both its microhabitat selection and its ability to thrive in non-optimal microsites where freezing risk is high. This shrub has been observed germinating successfully at high elevation sites and in other microhabitats where adults do not thrive (Auffret et al. 2010). As an argument towards its opportunism, *V. myrtillus* is known to be relatively responsive to environmental change: germination occurs significantly earlier with summer warming, budbreak is significantly earlier and growth ring width increases with warming, particularly in hot summers (Milbau et al. 2009, Prieto et al. 2009, Rixen et al. 2010). As a result, however, it is also known to be negatively impacted by certain site selection: leaf expansion and flowering is reduced by freezing events, and freezing resistance is decreased under

soil warming (Martin et al. 2010, Gerdol et al. 2013). Its plasticity and successful establishment on early-exposure microsites, despite its poor resistance and thus high likelihood of exposure to leaf-lethal spring freezing, suggests *V. myrtillus* establishment and growth represents an opportunistic response to periodic favourable conditions. Spring freezing causes aboveground damage, especially to leaf tissue, but is generally thought not to kill the species or control its survival at the population level (Körner and Larcher 1988, Taschler and Neuner 2004); *V. myrtillus* has been shown to regenerate leaf tissue lost to spring freezing damage (Wipf et al. 2009). However, repeated freezing events, especially after leaf maturity, when shrubs have already invested significant resources in leaf development, could potentially irreparably damage the leaf crop. Repeated seasons of freezing could then fatally deplete stored resources (Molau 1997).

Spring freezing damage has been shown to occur in wood and leaf tissue of *V. myrtillus* and *V. uliginosum* at treeline below our elevation gradient (Anadon-Rosell unpublished data, Rixen et al. 2012). It is thus very likely a factor at higher elevations, especially since microhabitat-driven snowmelt patterns expose many high-elevation ridges from snow at similar times or earlier than lower elevation sites. The temporal and spatial restriction of episodic cold events (only occur in May and June on ridge microhabitats) is relevant, as it indicates that only certain shrub communities risk damage. This trend has been observed in alpine wildflowers and dwarf shrubs growing in early snowmelt sites: earlier snowmelt leads to greater frequency of spring freezing events and significant damage to buds and leaf tissues, damage that is not sustained by plants growing in later snowmelt microsites (Inouye 2008, Wipf et al. 2009). This is a critical point, as snowmelt timing is significantly advancing in this and many other alpine communities (e.g. Inouye 2000, Inouye 2008, Rixen et al. 2012), and despite local warming air temperatures over the multi-decadal record (Rixen et al. 2012), there is no evidence that lethal

freezing events are in decline. Indeed, our extrapolation of spring cold event frequency may even be conservative, since it is determined from ambient air temperature, when radiative cooling is known to lower leaf surface temperatures by as much as 5K on clear nights (Jordan and Smith 1995). Ultimately, however, this extrapolation illustrates the increasing risk of leaf damage with elevation, with earlier snowmelt, and, to a lesser extent, through time. Wipf et al. (2009) referred to the paradox of a cold spring in a warmer world, which our results support: spring freezing events are not becoming rarer, and they may have a strong influence on individual shrub performance and thus community structure at high elevation and early exposure sites. We can also speculate that ridge communities may continue to remain more vulnerable than snowbed communities, as there is evidence that climate warming accelerates snowmelt on ridge microsites, while late snowbeds may remain relatively static (Kudo and Hirao 2006).

### *Conclusion*

Earlier snowmelt is predicted in many alpine ecosystems, and this abiotic change has critical implications for shrub community performance. In this and other studies cited, a longer growing season, driven by accelerated snowmelt, both fails to enhance growth, and increases risk of exposure to damaging spring freezing events at leaf maturity. This suggests that the detrimental effects associated with an extended growing season may outweigh the benefits for some alpine shrub species. As snowmelt timing accelerates, spring freezing events, which are not in decline despite warming in this system, are likely to become more common, especially in high-elevation and early-snowmelt communities.

### *Acknowledgements*

We wish to acknowledge and thank Armando Lenz (University of Basel) for assistance provided during the laboratory freezing experiments, to Felix Prahl and Yves Bötsch for their help during field sampling, to Guenther Klonner for data extraction assistance, and to two anonymous reviews for improvements to the manuscript. This project was made possible by funding from the Swiss National Science Foundation (grant CRSI33\_130409/1).



Table 1: Elevation and snowmelt day of paired ridge (R, N=4) and snowbed (S, N=4) sampling sites and average daily minimum, maximum, and mean air temperatures (°C at 2 m) during the 7-day period prior to biomass sampling (June 16-June 23 2012)

	<b>Low</b>	<b>Low-intermediate</b>	<b>High-intermediate</b>	<b>High</b>
Elevation (m)	2109	2260	2372	2510
Snowmelt (day of year)	R=148 S=162	R=132 S=145	R=158 S=170	R=130 S=159
Minimum Temp (°C)	8.71	7.74	7.04	6.14
Maximum Temp (°C)	18.23	17.26	16.56	15.66
Mean Temp (°C)	13.10	12.13	11.43	10.53

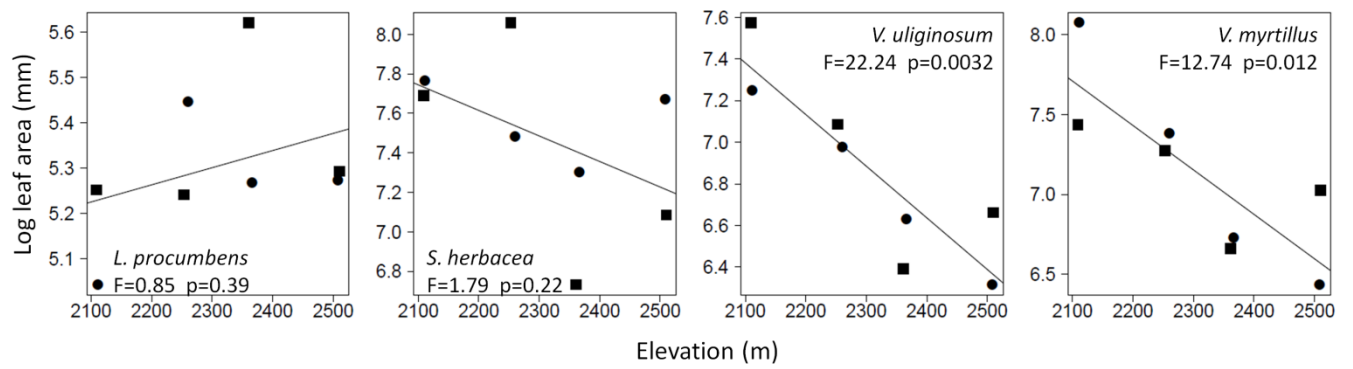


Figure 1: Mean leaf area along elevational gradient in *L. procumbens*, *S. herbacea*, *V. uliginosum* and *V. myrtillus*. Squares represent ridges and circles represent snowbed microhabitats. F- and p-values refer to the linear regressions for each species (black line). Note log scale used on y-axis and different y-axes for the different species.

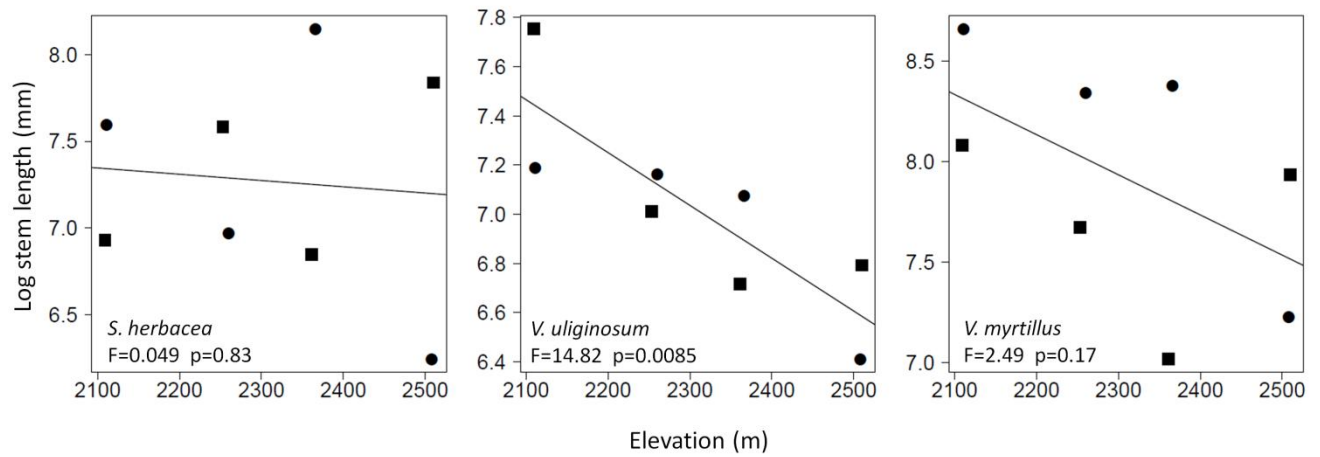


Figure 2: Mean stem length along elevational gradient in *S. herbeacea*, *V. uliginosum* and *V. myrtillus*.

Squares represent ridges and circles represent snowbed microhabitats. Stem length excluded for *L.*

*procumbens* due to uncertainty in determining total stem length in samples. F- and p-values refer to the linear regressions for each species (black line). Note log scale used on y-axis and the different y-axes for the different species.

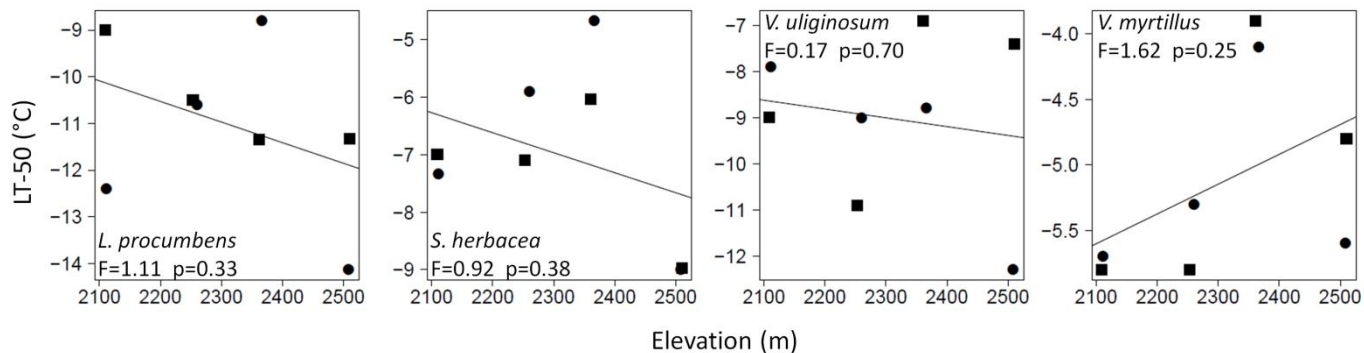


Figure 3: LT-50 along elevational gradient in *L. procumbens*, *S. herbacea*, *V. uliginosum* and *V. myrtilus*. Squares represent ridges and circles represent snowbed microhabitats. F- and p-values refer to the linear regressions for each species (black line). Note the different y-axes for the different species.

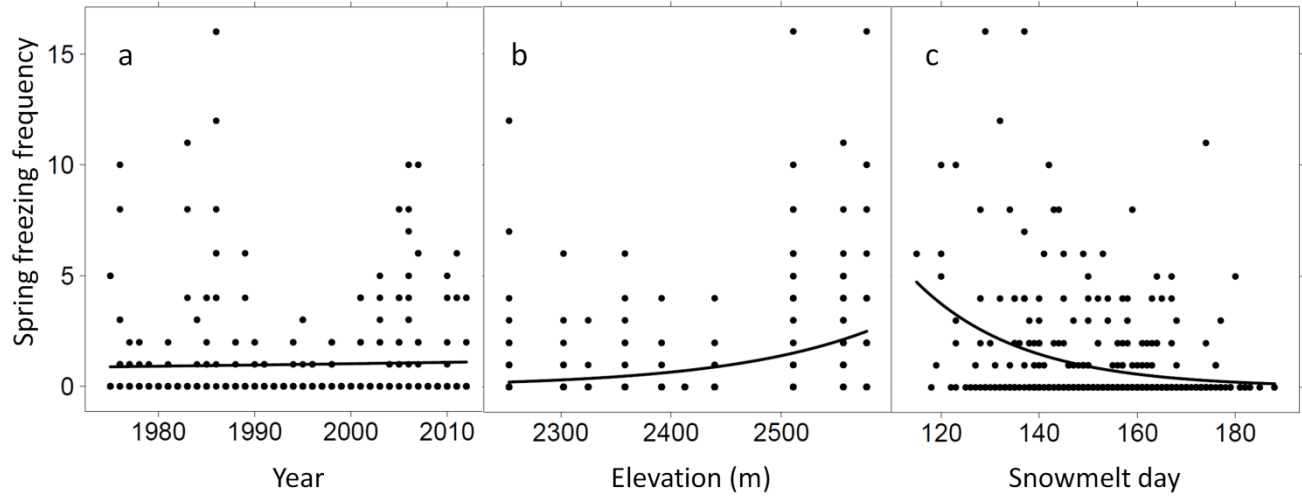
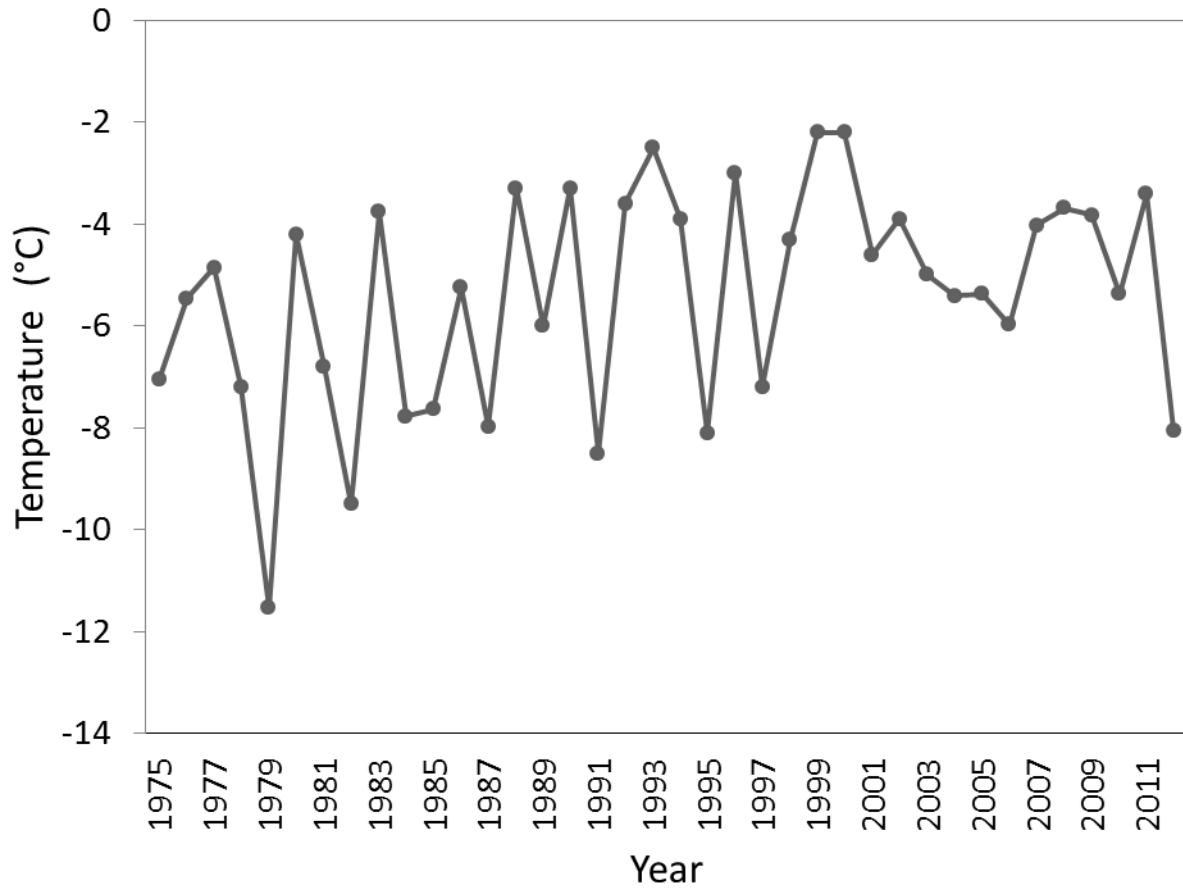


Figure 4: Modelled frequency of potentially lethal spring freezing events between 1975 and 2012 (37 years) for *V. myrtilus* in dependency of year (panel A), elevation (panel B) and snowmelt day (panel C).

## Supplemental Information

Supplemental Table 1: General linear models results for snowmelt timing, June and season soil temperature, shrub growth traits, freezing resistance and extrapolated spring freezing events along the elevational and snowmelt timing gradients

Response variable	Explanatory variable 1	F / t value + p-value	Explanatory variable 2	F / t value + p-value
<b>Snowmelt timing (day of year)</b>	Elevation (m)	F=0.24 p=0.62	Microhabitat type (ridge/snowbed)	F=20.1 p<0.0001
<b>June growing degree days (GDD)</b>	Elevation (m)	t=-1.96 p=0.059	Microhabitat type (ridge/snowbed)	F=17.8 p=0.0002
<b>Growing season growing degree days (GDD)</b>	Elevation (m)	t=-1.84 p=0.075	Microhabitat type (ridge/snowbed)	F=1.2 p=0.26
<b>Mean June temperature (°C)</b>	Elevation (m)	t=-5.17 p<0.0001	Microhabitat type (ridge/snowbed)	F=1.7 p=0.20
<b>Mean growing season temperature (°C)</b>	Elevation (m)	t=-2.49 p=0.018	Microhabitat type (ridge/snowbed)	F=13.2 p=0.001
<b>Mature leaf area, <i>V. myrtillus</i></b>	Elevation (m)	F=12.74 p=0.012	Snowmelt timing (day of year)	F=0.18 p=0.69
<b>Mature leaf area, <i>V. uliginosum</i></b>	Elevation (m)	F= 22.42 p=0.0032	Snowmelt timing (day of year)	F=0.38 p=0.56
<b>Mature leaf area, <i>S. herbacea</i></b>	Elevation (m)	F=1.79 p=0.23	Snowmelt timing (day of year)	F=0.19 p=0.68
<b>Mature leaf area, <i>L. procumbens</i></b>	Elevation (m)	F=0.8456 p=0.39	Snowmelt timing (day of year)	F=0.016 p=0.90
<b>Mean stem length, <i>V. uliginosum</i></b>	Elevation (m)	F= 14.82 p=0.0085	Snowmelt timing (day of year)	F=0.018 p=0.89
<b>Mean stem length, <i>V. myrtillus</i></b>	Elevation (m)	F=2.49 p=0.17	Snowmelt timing (day of year)	F=0.075 p=0.79
<b>Mean stem length, <i>S. herbacea</i></b>	Elevation (m)	F=0.049 p=0.83	Snowmelt timing (day of year)	F=0.054 p=0.82
<b>Freezing resistance (LT-50) <i>L. procumbens</i></b>	Elevation (m)	F=1.11 p=0.33	Snowmelt timing (day of year)	F=0.066 p=0.81
<b>Freezing resistance (LT-50) <i>S. herbacea</i></b>	Elevation (m)	F=0.92 p=0.38	Snowmelt timing (day of year)	F=1.31 p=0.30
<b>Freezing resistance (LT-50) <i>V. uliginosum</i></b>	Elevation (m)	F=0.17 p=0.70	Snowmelt timing (day of year)	F=0.0024 p=0.96
<b>Freezing resistance (LT-50) <i>V. myrtillus</i></b>	Elevation (m)	F=1.62 p=0.25	Snowmelt timing (day of year)	F=1.21 p=0.32
<b>Spring freezing events <i>V. myrtillus</i></b>	Elevation (m)	t=6.89 p<0.0001	Snowmelt timing (day of year)	t=-7.16 p<0.0001



**Supplemental Figure 1:** Spring (May-June) air temperature minima after snowmelt at Jakobshorn climate station (2090 m asl) between 1975 to 2012.

## Chapter 6

### Some further publications

This project was carried out in extensive collaboration with two other PhD students, Janosch Sedlacek (University of Konstanz, Germany) and Andrés Cortés (University of Uppsala, Sweden), who both co-authored all the manuscripts in this doctoral thesis. Below I list the abstracts for their published manuscripts for which I was involved as a co-author.

Sedlacek, JF, Bossdorf, O, Cortés, AJ, Wheeler, JA, van Kleunen, M. 2014. **What role do plant-soil interactions play in the habitat suitability and potential range expansion of the alpine dwarf shrub *Salix herbacea*?** Basic and Applied Ecology, 15: 305-315

Mountain plants may respond to warming climates by migrating along altitudinal gradients or, because climatic conditions on mountain slopes can be extremely locally heterogeneous, by migrating to different microhabitats at the same altitude. However, in new environments, plants may also encounter novel soil microbial communities, which might affect their establishment success. Thus, biotic interactions could be a key factor in plant responses to climate change. Here, we investigated the role of plant-soil feedback for the establishment success of the alpine dwarf shrub *Salix herbacea* L. across altitudes and late- and early snowmelt microhabitats. We collected *S. herbacea* seeds and soil from nine plots on three mountain-slope transects near Davos, Switzerland, and we transplanted seeds and seedlings to substrate inoculated with soil from the same plot or with soils from different microhabitats, altitudes and mountains under greenhouse conditions. We found that, on average, seeds from higher altitudes (2400-2700 m) and late-exposed snowbeds germinated better than seeds from lower altitudes (2200-2300m) and early-exposed ridges. However, despite these differences in germination,



growth was generally higher for plants from low altitudes, and there were no indications for a home-soil advantage within the current range of *S. herbacea*. Interestingly, seedlings growing on soil from above the current altitudinal distribution of *S. herbacea* grew on average less well than on their own soil. Thus, although the lack of a home-soil advantage might be beneficial for *S. herbacea* in a changing environment, migration to habitats beyond the current altitudinal range might be limited, probably due to missing positive soil-feedback.

Cortés, AJ, Waeber, S, Lexer, C, Sedlacek, J, Wheeler, JA, van Kleunen, M, Bossdorf, O, Hoch, G, Rixen, C, Wipf, S, Karrenberg, S. 2014. **Small-scale patterns in snowmelt timing affect genetic diversity and gene flow in the alpine dwarf shrub *Salix herbacea***. *Heredity*, 113: 233-239.

Current threats to biodiversity, such as climate change, are thought to alter the within-species genetic diversity among microhabitats in highly heterogeneous alpine environments. Assessing the spatial organization and dynamics of genetic diversity within species can help to predict the responses of organisms to environmental change. In this study, we evaluated whether small-scale heterogeneity in snowmelt timing restricts gene flow between microhabitats in the common long-lived dwarf shrub *Salix herbacea* L. We surveyed 273 genets across 12 early- and late-snowmelt sites (i.e. ridges and snowbeds) in the Swiss Alps for phenological variation over two years and for genetic variation using seven SSR markers. Surprisingly, phenological differentiation triggered by differences in snowmelt timing did not correlate with genetic differentiation between microhabitats. On the contrary, extensive gene flow appeared to occur between microhabitats, and slightly less extensively among adjacent mountains. However, ridges

exhibited significantly lower levels of genetic diversity than snowbeds and patterns of effective population size ( $N_e$ ) and migration ( $N_e m$ ) between microhabitats were strongly asymmetric, with ridges acting as sources and snowbeds as sinks. Since no recent genetic bottlenecks were detected in the studied sites, this asymmetry is likely to reflect current meta-population dynamics of the species dominated by gene flow via seeds rather than ancient re-colonization after the last glacial period. Overall, our results indicate that seed dispersal prevents snowmelt-driven genetic isolation and snowbeds act as sinks of genetic diversity. Disentangling the consequences of this asymmetric gene flow and diversity levels is essential for understanding community responses to climate change.

## Chapter 7

### Summary and conclusions

#### *Chapter 2: Fitness and performance under early snowmelt and warming*

In our space-for-time substitution study, snowmelt timing, with its colder temperatures at earlier snowmelt and warmer late-melting sites, represented the primary driver of trait variation in *Salix herbacea*. Early snowmelt generally led to a reduction in performance in functional and fitness traits. We found that under early snowmelt conditions, phenological development began earlier but lasted longer, that flowering increased but fruit set declined, that stem density decreased, C-supply remained unchanged (as derived from non-structural carbohydrate stores in end-of-season wood tissue), and that the probability of leaf damage by herbivores and phytopathogens increased significantly. Lower elevation microsites, with their warmer temperatures, were associated with increased fruiting but lower stem density.

*Salix herbacea* was not able to translate a longer growing season into enhanced growth (higher stem density) or increased C assimilation (higher non-structural carbohydrate accumulations). Our results suggest that under the accelerated spring snowmelt projected for the Swiss Alps, we will likely see reduced fitness (lower sexual and clonal production) and greater damage in *S. herbacea* and potentially in other low-lying dwarf shrubs as well. We suggest that low temperature events in the early growing season, more common as snowmelt shifts earlier in the season, may be the primary mechanism driving reductions in fitness traits. In addition, increased probability of exposure to leaf damage agents under earlier snowmelt also has likely fitness implications. Warming temperatures led to increased fruiting but lower clonal production, suggesting that the potential benefits of warming may not outweigh the detrimental effects of

earlier snowmelt for this species. We conclude that earlier snowmelt timing likely represents a critical ecosystem shift for this species, with negative implications for performance, fitness and potentially even persistence. These results may also have strong implications for other prostrate shrub species in both arctic and alpine tundra ecosystems, which are low-lying and may also be tightly ecologically synchronized with snowmelt timing. Reduced snowpacks and earlier spring melt-out, as a result of global warming, may represent one of the principal mechanisms driving the general reductions in dwarf shrub communities observed throughout the tundra biome.

### *Chapter 3: Trait plasticity and changes in snowmelt timing*

The results from the reciprocal transplant experiment were broadly consistent with the larger-scale space-for-time substitution, indicating that snowmelt timing, with its associated environmental conditions, is an important driver of functional response for multiple traits in *S. herbacea*. In our reciprocal transplant experiment, phenological timing, leaf size and leaf damage probability were all strongly influenced by the destination microhabitats when *Salix herbacea* turfs were transplanted. Earlier snowmelt at the destination microhabitat led to earlier but slower leaf expansion, smaller leaf size and increased probability of leaf damage. Flowering and stem density remained relatively fixed two years after transplant, consistently demonstrating only origin microhabitat effects, with lower stem density and higher flowering proportion in *S. herbacea* turfs originating from early snowmelt sites. Selection gradient analysis indicated that neither development time to leaf expansion or leaf damage probability influenced fitness (change in stem number or flowering probability) in the short term. However, the same analysis suggested that smaller leaf size was associated with reduced flowering probability on early-exposure sites.

Finally, *S. herbacea* did not demonstrate a home-site advantage to local snowmelt conditions, likely as a result of strong gene flow between microhabitats.

*Salix herbacea* responded to new micro-environmental conditions under altered snowmelt timing through plastic adjustment of leaf size, and changes in phenological timing as driven by new temperature conditions. We found a positive selection for larger leaf size on early exposure microhabitats, in that larger leaves were associated with higher flowering probability. However, the environmentally-driven plastic shift to smaller leaf sizes on early snowmelt sites suggests that this could lead to flowering reductions under accelerated spring snowmelt conditions. Under early snowmelt, the damage probability increased, but in this study, a selection gradient analysis showed no evidence that leaf damage caused flowering reductions. However, this may have been an effect of a smaller sample size with lower flowering, as the larger *S. herbacea* data set generally demonstrated reduced female flowering in the season following damage.

There was no evidence of a home site advantage to snowmelt timing, potentially as a result of high gene flow via seeds between subpopulations in phenologically disparate microhabitats. As it not strongly adapted to current snow conditions, we speculate that *S. herbacea* may be able to maintain itself under changing spring snowmelt conditions, but the lack of small-scale adaptive divergence between subpopulations could possibly mean *S. herbacea* may not have the potential to adapt to new extremes in snowmelt timing.

#### *Chapter 4: Community facilitation under climate change*

In our neighbour removal experiment, fruiting phenology and herbivory probability in *Salix herbacea* were directly impacted by neighbor removal, with more rapid development of fruit and higher herbivory when neighbouring vegetation was removed. Further, with neighbor

removal, leaf size was reduced on earlier snowmelt microhabitats, as indicated by the shift from competitive to facilitative relative neighbor effects, and phytopathogen damage probability increased with elevation. Finally, neighbours may have indirectly improved fitness in *S. herbacea*, as leaf damage in the previous seasons generally reduced flowering probability in females in the following growing seasons.

Plant-plant interactions between *S. herbacea* and their neighbours were generally in line with the predictions of the stress gradient hypothesis, as most neighbour interactions were facilitative at the more stressful extremes of their distributions at high elevations and early exposure sites. Neighbours never directly reduced sexual or clonal production in *S. herbacea*, may have indirectly increased flowering probability through damage sheltering, and only appeared to compete directly with this species in by reducing leaf size in late-lying snowbeds. We suggest that under accelerating snowmelt conditions, community facilitation may play an increasingly important role in mediating the functional response of *S. herbacea* to climate change. Under the likely more stressful early-snowmelt conditions, facilitative neighbor interactions may even act to buffer the declining fitness and performance that we predict for *S. herbacea* under early snowmelt and changing climates. While competition for light from a taller shrub canopy is discussed as a mechanism reducing dwarf shrub communities in arctic and alpine tundra, facilitative interactions from taller neighbours must also be considered as a potential positive interaction ameliorating performance in low-lying shrub species. These facilitative interactions may become particularly important as global climate change may drive significant increases in local environmental stress.

*Chapter 5: Spring frost vulnerability under climate change*

The examination of freezing resistance in four alpine shrub species revealed that LT-50 for mature leaf tissue was species-specific, but did not vary for any species along the elevation or snowmelt gradients. *Vaccinium myrtillus* had the highest vulnerability to spring frost events, with leaf tissue death occurring at  $-5.1^{\circ}\text{C}$ . Further, this species grew in microhabitats that were characterized by relatively frequent spring frost events. Spring frost events were extrapolated from the long-term temperature record. For *V. myrtillus* and to a lesser extent for *S. herbacea*, but not for the other investigated species, potentially lethal spring frost events occurred more frequently with early snowmelt and with increasing elevation. Despite warming air temperatures, spring frost events did not decrease, and even increased slightly over the 37-year climate record.

All four alpine shrub species examined in this study failed to capitalize on a longer growing season with enhanced growth, a result consistent with the results from both the space for time substitution and the reciprocal transplant for *S. herbacea*. We speculate that early-season frost events leading to leaf death are at least partially responsible for this failure, since the early snowmelt that characterizes a longer growing season led to increasing exposure to spring freezing events for *V. myrtillus* and to a lesser extent, *S. herbacea*. Spring frosts may become an increasingly important damage agent under early snowmelt in arctic and alpine tundra communities, and, in the long term, will likely reduce performance and may even remove some shrub species from early-exposure microhabitats.

## Main conclusions

1. Early snowmelt as a consequence of climate change in alpine ecosystems represents a significant and detrimental environmental change for prostrate shrub *Salix herbacea* due to exposure to increasing environmental stress factors such as spring frost events, herbivory, and phytopathogens. In addition to reducing the current leaf crop, herbivory and phytopathogen damage can lead to reductions in flowering probability in the following summers. Thus, accelerated spring snowmelt potentially represents a critical mechanism reducing growth and performance for alpine and arctic dwarf shrubs, due to their close ecological synchronization with snowmelt timing.
2. *Salix herbacea* traits may be strongly responsive to changing climatic conditions. While climate warming may be associated with enhanced fruit production, under early snowmelt *S. herbacea* demonstrate reduced fruit set, have lower stem numbers, show slower phenological development and are more likely to suffer biotic and abiotic damage.
3. Plastic adjustments in response to changes in snowmelt timing, such as smaller leaf size on early-exposure microhabitats may be potentially maladaptive, since larger leaves are associated with higher fitness under early snowmelt conditions.
4. A lack of home-site advantage to local snowmelt conditions, however, suggests *S. herbacea* may not be strongly specialized, and may thus be able to maintain their population under novel snowmelt conditions.
5. The detrimental effects of early snowmelt for *S. herbacea* may be mediated to some extent by facilitative interactions by the surrounding plant community, through enhanced leaf size and protection from herbivores and phytopathogens. Increasing environmental stress may also increase the importance of neighbour facilitation for *S. herbacea* and other arctic and alpine dwarf shrubs.



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*All this he saw, for one moment breathless and intense, vivid on the morning sky; and still, as he looked, he lived; and still, as he lived, he wondered.*

— Kenneth Grahame, *The Wind in the Willows*

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**PhD in Botany / Plant Ecology (February 2011 – October 2014)**

Institute of Botany, University of Basel, Basel CH

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Thesis title: The snow and the willows: ecological responses of the alpine dwarf shrub *Salix herbacea* to climate change

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**Master of Science in Biology (May 2007 – May 2009)**

Department of Biology, Memorial University, St. John's CAN

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**Research Experience**

**PhD Researcher**

Swiss Federal Institute for Snow and Avalanche Research, Davos CH

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February 2011 – September 2014

- Collaborated on design and implementation of a large-scale, 3-year field experiment monitoring growth, phenology, reproduction, non-structural tissue carbohydrates, herbivory, freezing resistance and neighbour interactions in 480 alpine dwarf shrub *Salix herbacea* clones along elevational transects and snowmelt gradients
- Collaborated on design and implementation of a field reciprocal transplant experiment between snowmelt microhabitats to examine local adaptation and trait plasticity in 350 *S. herbacea* clones
- performed non-structural carbohydrate concentration analyses on leaf and wood tissue in the laboratory
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- Managed and analysed multi-year data sets using R
- Prepared first-authorship and co-authored manuscripts
- Co-supervised 3 M.Sc students

### **Research Associate**

Memorial University, St. John's CAN

August 2007 - August 2010

- Collaborated on design and helped co-ordinate an ongoing ecological / climate monitoring project in the Torngat Mountains National Park Reserve, northern Labrador
- Established open top warming chambers and control plots, carried out vegetation surveys, and collected and identified vascular plants, mosses and lichens

### **M.Sc researcher**

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May 2007 - May 2009

- Designed and implemented a two-year research project examining early black spruce survival in the alpine treeline of the Mealy Mountains, Labrador as part of a landscape-scale multi-disciplinary initiative under the funding of IPY-Canada and PPS-Arctic
- principal contributor of initial proposal, experimental design and implementation of field methods, data analysis and the preparation of thesis manuscript

### **Fisheries Field Technician**

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- Assisted with beach surveys and GPS mapping of capelin spawning beds, underwater video mapping of capelin spawning beds, adult and larval lobster surveys and beach seining of subtidal fish assemblages

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### **Teaching Experience**

#### **Laboratory Instructor, Biological Sciences**

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Taught laboratory component of first and second-year biology core courses (Organisms and the Environment, Introduction to Ecology)

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January 2010 – August 2010

Collaborated on the development of a new lab curriculum and lab manual material for new 3<sup>rd</sup> and 4<sup>th</sup>-year university Biology courses (Terrestrial Ecology and Aquatic Ecology)

### **Graduate Teaching Assistant**

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Acting as teaching assistant for first, second and fourth year biology courses (Introduction to Biology I and II, Introduction to Ecology, Modern Biology and Human Society, Community and Ecosystem Ecology)

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#### Co-authorship manuscripts

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### **Manuscripts in progress**

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Trant, A, Lewis, K, Cranston, BC, Wheeler, JA, Jameson, R, Jacobs, J, Hermanutz, L, Starzomski, B. Discontinuous changes in plant communities across a subarctic-alpine treeline. **In review**, *Plant Ecology and Diversity*

Myers-Smith, I, Elmendorf SC, Beck, PSA, Wilmking, M, Hallinger, M, Blok, D, Tape, KD, Rayback, SA, Macias-Fauria, M, Forbes, BC, Speed, JDM, Boulanger-Lapointe, N, Rixen, C, Lévesque, E,

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### **Conference presentations**

Wheeler JA, Hoch G, Wipf S, Rixen C. 2014. Early spring snowmelt reduces performance in the alpine shrub *Salix herbacea*: lessons from a three-year space for time substitution. Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Hildesheim, GER.

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Wheeler JA, Hoch G, Wipf S, Rixen C. 2013. Increased spring freezing vulnerability for alpine shrubs under early snowmelt. International Tundra Experiment ITEX - More than 20 years of tundra vegetation change research, Bergün, CH  
**Winner, Best Student Talk, 2<sup>nd</sup> prize**

Wheeler JA, Hoch G, Wipf S, Rixen C. 2013. Longer, warmer, less productive: early snowmelt reduces performance of alpine shrub *Salix herbacea*. Swiss Geosciences Meeting 2013, Lausanne, CH

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Wheeler JA Hermanutz L, Marino PM. 2009. How does seedbed affect black spruce success at the Labrador treeline? Biology Graduate Student Symposium, Memorial University, St. John's CAN  
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Wheeler JA, Hoch G, Wipf S, Rixen C. 2014. Accelerated spring snowmelt slows phenological development in dwarf shrub *Salix herbacea*. In European Geoscience Union General Assembly 2014, Vienna, AUS

Wheeler JA, Hoch G, Wipf S, Rixen C. Reduced alpine shrub performance and freezing risk under early snowmelt. In Mountains Under Watch 2013, Aosta, ITA

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Trant, A, Bell, T, Hermanutz, L, Jacobs, J, Laroque, C., Lewis, K, Simms, A, Simms, E, Bartlett, Z, Chan, S, Cranston, B, Jameson, J, Morrison, H, Sutton, E, Trindade M and Wheeler, JA. 2010. A (complicated) story of treeline dynamics in the Mealy Mountains, Canada. In International Polar Year Oslo Science Conference, Oslo, NOR. **Winner, Best Poster, Polar Ecosystems and Diversity**

Wheeler, JA, Cranston, B, Hermanutz, L, Simms, A. 2009. Is there evidence of facilitation in Arctic tundra? In Canadian Botanical Association 45<sup>th</sup> Annual Meeting: Plants on the Periphery, Wolfville, CAN.

Wheeler, JA, Hermanutz, L, Marino, P. 2009. Seedbed facilitation in the forest-tundra ecotone, Mealy Mountains, Labrador, Canada. In Canadian Society for Ecology and Evolution, Halifax, CAN.

Cranston, B, Koncz, P, Wheeler, JA, Hermanutz, L, Marino, P, Upshall, M, Simms, A, Chan, S, Jacobs, J. 2008. Impacts of climate change on the tundra ecosystem of the Torngat Mountain National Park Reserve, Labrador. In CiCAT Annual General Meeting: Canadian Arctic Tundra Ecosystems in a Changing Climate, Ottawa, CAN.

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Swiss Federal Institute for Snow and Avalanche Research, Davos CH  
September 2012-August 2014

Public Tour Guide

- worked with student and adult tour groups to publicize scientific research at the institute in an accessible and friendly manner

Biology Graduate Students Association, St. John's CAN  
December 2007-June 2009

Professional Development Coordinator

- organized new graduate student orientations, academic development seminars, and assisted planning social events

- liaised between graduate students and Biology Department to assist with resolving academic and financial issues

-served on Graduate Studies Committee as student representative



Biology Graduate Student Symposium, St. John's CAN

January 2009-April 2009

Organizing Committee Chairperson

- responsible for organizing schedule, recruiting outside keynote speaker, designing advertising posters and print media, securing financing and sponsorship, and general logistics for a University-level outreach event

Let's Talk Science Canada, St. John's CAN

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Student Outreach Volunteer

- worked with grade-school students to demonstrate basic principles of science in a fun and interactive environment