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Bud freezing resistance in alpine shrubs across snow depth gradients
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HIGHLIGHTS

We analysed early spring freezing resistance for the buds of eight alpine shrubs.
Freezing resistance was related to plant phenology and preferences for snow cover
Plants from shallower snow had higher sugar concentrations and were hardier
Snow depth affects the physiological hardening of plants during early spring
Changes in snow cover can be a relevant driver of plant responses to climate change

Abstract

Despite the importance of spring freezing events for alpine species distribution, few studies have analysed the response of alpine shrub species to early spring freezes. It is also not known how snow cover gradients influence the process of de-hardening between individuals of the same species and their vulnerability to early spring frosts. We analysed early spring freezing resistance for the buds of eight alpine Ericaceae shrubs growing at 1 m snow depth at treeline in the Swiss Alps. Moreover, buds of *Rhododendron ferrugineum* and *Loiseleuria procumbens* were analysed for freezing resistance and sugar content along a snow depth gradient. The LT₅₀ (lethal temperature for 50% of samples) of the eight species ranged from -25.1 ± 1.6 °C (*Vaccinium vitis-idaea*) to -11.1 ± 1.2 °C (*Vaccinium uliginosum*), with differences being related to the phenological stage in addition to shrub preferences for contrasting snow cover microsites. Although the effect of snow depth on the freezing resistance of plants was not significant, samples collected from 1 m and 1.5 m snow depth tended to be more vulnerable to freezing, particularly *L. procumbens*. Buds collected at shallower snow depths had higher sugar concentrations, indicative of their stronger physiological hardening. Consequently, we conclude that differences in snow cover may significantly affect the physiological hardening of plants during the onset of spring. Individuals growing at less than 0.5 m snow cover are hardier, i.e. show moderately higher freezing resistance than individuals from snow banks. Snow cover is a highly important aspect of climate change, and freezing resistance in alpine plants with respect to snow conditions can be a relevant driver of plant responses to climate change.

Keywords: plant phenology, frost hardiness, Ericaceae, sugars, treeline, winter ecology.

1. Introduction

Freezing temperatures are a main selection filter and a decisive adaptive force in alpine and arctic environments. This is particularly true in the alpine zone above treeline,

where air temperatures become more extreme and limiting, and vegetation is dominated by shrubs (Inouye, 2000; Körner, 2003). In alpine environments, freezing temperatures may occur at any time of the year, but spring freeze events have been identified as the main selective force determining the distribution of tree species (Lenz et al., 2013). Although spring freezes rarely kill individuals, differences in spring freezing resistance may affect the ability of plants to grow, take up nutrients and water and compete with neighbouring species (Bokhorst et al., 2008; Rixen et al., 2012).

In arctic and alpine environments, snow is a major determinant of the annual life cycle of plants. Plants growing on snow-free microsites, where temperatures may fall below -30°C during winter, need to be more frost resistant than those growing under a deep insulating snow layer, where temperatures rarely fall below -5°C (Larcher et al., 2010). In spring, a difference in snow cover duration driven by variation in snow melt timing will in turn lead to a difference in phenology (Keller and Körner, 2003; Wipf and Rixen, 2010). When warming starts in early spring, plants growing at more exposed locations may de-harden earlier than plants from areas still covered in snow, and thus become more vulnerable to freezing (Taulavuori et al., 2004; Inouye, 2008; Skre et al., 2008; Pagter and Arora, 2013). Presumably, de-hardening in early spring would be attenuated in species with a preference for snow-free microsites, which have been found to retain higher frost resistance than snow-bed species throughout the year (Bannister et al., 2005). Accordingly, leaves of species preferentially occurring on exposed ridges and snow-free sites are more resistant to late spring freezes than those of typical snowbed-species (Wheeler et al., 2014). In addition, earlier-flushing tree species are more freezing resistant than later-flushing tree species, counterbalancing the lower temperatures earlier in the season (Lenz et al., 2013); this represents a possible mechanism for shrubs as well (but see Taulavuori et al., 2004 and Skre et al., 2008). Similarly, the ability to advance phenological development in early spring, while maintaining a certain resistance to spring freezes and a specific re-hardening capacity, may vary between species, and likely relates to their preferences for snow-cover microsites. Despite the role of early spring freezing events in structuring alpine and arctic species distribution, few studies have analysed the response of alpine species to early spring freezes (but see Taschler et al., 2004). This lack of knowledge is particularly critical for the case of alpine shrubs thriving above the timberline.

Plants have adopted many physiological, ultrastructural, and morphological adjustments associated with freezing resistance (for reviews, see Sakai and Larcher, 1987, Larcher, 2005, Wisniewski et al., 2004). In many cases, low molecular-weight carbohydrates (mono- and disaccharides, raffinose family oligosaccharides, and polyols) are increased during hardening (Larcher, 2005). Due to their osmotic function, soluble sugars mediate effects of intracellular desiccation during the freezing process (Kandler et al., 1979), and are involved in protecting protein structures and cell membranes (Sung et al., 2003). These compounds are produced at the end of autumn, and their concentrations remain high in frost-hardened plants until the beginning of spring de-hardening (Larcher, 2005). Therefore the analysis of non-structural carbohydrate concentrations, especially of soluble sugars, may provide valuable information on the changes in the freezing resistance of plants.

The interplay between snow cover preference, phenology and freezing resistance at different times of the year determines the probability of freezing damage in alpine and arctic plants, which can affect their performance and competitive ability within the community (Inouye, 2000; Inouye, 2008; Rixen et al., 2012). The aim of this study was to compare the bud freezing resistance of eight Ericaceae species which demonstrate different phenological timing in spring and ecological preferences along snow depth

gradients, and to explore the effect of snow cover depth on the sensitivity to freezing of alpine sub-shrubs in early spring. We hypothesized that: i) species characteristic to microhabitats with thick snow will be more vulnerable to early spring freezing than species typical to exposed sites, even when growing under similar snow cover depth. Within a given species ii), individuals growing in areas with thicker snow cover will be less physiologically hardened and hence more vulnerable to freezing damage than uncovered individuals or plants covered by a thin snow layer (given that temperatures outside the protection of snow are generally colder than below snow during winter and early spring in this alpine environment) and iii) the pattern of hardening across the snow gradient will be reflected in the tissue concentrations of soluble sugars, indicating the physiological adaptation to freezing temperatures.

2. Materials and methods

2.1. Study species and sites

We selected eight Ericaceae species for this study. Each of the shrubs are widely distributed throughout Europe but show differences in their ecological optimum along snow depth gradients (Table 1). We determined the shrub preferences along the snow depth gradient according to common phytosociological knowledge and our own field observations (Ellenberg, 1988; Körner, 2003; Rixen et al., 2010). Except for *E. carnea*, which was harvested from an alpine tundra site on calcareous rock in the neighbouring Sertig valley (9°85'E, 46°71'N, between 2000 and 2100 m a.s.l.), we collected all shrubs in alpine tundra at the Stillberg treeline experimental site (9°52'E, 46°46'N; between 2100 and 2250 m a.s.l.). Both sites are located in Davos, Central Alps, SE Switzerland.

2.2. Field design and plant harvest

We harvested plants on May 2nd, 2012, when most of the area was covered in snow, apart from wind-swept ridges that had been exposed from snow a few days previously. The winter 2011-2012 was exceptionally snow-rich in the study area (Wheeler et al., submitted). Climatic conditions in the weeks prior to sampling were typical of winter (with permanent temperatures below 0°C); however, 5 days prior to sampling, above-freezing temperatures were recorded and water began to infiltrate the soil below the snow cover, indicating the start of snow melt (Fig. 1). Therefore, our sampling time coincided with the shift to spring environmental conditions.

At sites with a topographical gradient ranging from snow-exposed ridges to snow-filled bowls, we dug snow pits at approximately 0, 25, 50, 100, and 150 cm snow depth (measured with a probe) and collected branches of *Rhododendron ferrugineum* and *Loiseleuria procumbens* from a total of three locations for each species and snow depth. At additional random locations with approx. 1 m snow depth and separated by a minimal distance of 10 m, we collected branches of all available species (Table 1) until all species had been collected from three different snow pits. Branches, packed in plastic bags to prevent desiccation, were immediately stored in cooling boxes and kept at approx. 4°C until further processing in the laboratory. We collected all plant material between 09:00 AM and 12:00 PM. We sampled bud material for non-structural carbohydrates analyses from the same branches used for the freezing resistance assessments. We dried all buds sampled for chemical analyses at 75 °C for 2 days, ground the samples to fine powder in a mixer-mill (MM20, Retsch, Germany) and stored them well sealed and dry until analysis.

2.3. Freezing resistance tests

We determined freezing resistance at the freezing lab facility at the University of Basel (Basel, Switzerland) in the day following field collection. We selected at least three current-year shoots with living vegetative apical buds and detached from the same branch for each sample replicate. In *V. myrtillus* and *V. uliginosum*, which show sympodic growth (i.e. apical buds are aborted), we collected subapical buds. We placed samples in paper bags and wrapped them in paper and aluminum foil to prevent desiccation during freezing and to ensure a homogenous thermal environment during freezing cycles. Samples were kept in darkness at 4 °C until freezing, which we carried out within 24 h of collection. We implemented freezing conditions overnight. The freezing lab consisted of seven modified, computer-controlled commercial freezers, which allowed for a controlled cooling and re-thawing rate (described in Lenz et al., 2013). We applied seven different freezing temperature treatments concurrently, with three samples per species in each of the treatments (Lenz et al., 2013). Each of the seven freezers was programmed for a different final target temperature in order to create a temperature gradient spanning the species-specific critical range. Temperature inside each freezer was recorded using Pt-100 sensors inserted into the sample packages. After an initial reduction to 4 °C, we decreased the temperature within each freezer by 3 K h⁻¹ and then maintained the temperature at the target value for 4 h. This process of slow cooling helped reduce the possibility of either excessive supercooling or failure to nucleate (Sakai and Larcher, 1987), artifacts that have been observed for small, excised samples (Neuner et al., 1997). The span of target temperatures for the different freezers ranged from -10 °C to -37 °C, in steps of 4.5 K. Temperatures inside the freezers were slightly warmer than target temperatures and included -6.6 °C, -13.1 °C, -17.2 °C, -22.1 °C, -26.3 °C, -30.2 °C and -34.7 °C. After having been held at the target temperature for 4 h, we warmed the samples by 3 K h⁻¹ until they reached 4 °C. Samples were then kept in darkness at 4 °C. In addition to the seven freezers, we included a control (4 °C) and a maximum damage (-80 °C) treatment using a standard commercial unit, providing a total of nine temperature treatments.

2.4. Damage assessment

We determined bud damage by visual scoring, a basic yet reliable technique (e.g. Lenz et al., 2013; Obrist et al., 2001; Taschler and Neuner, 2004), by dissecting buds under the stereomicroscope within 8-10 hours after the completion of freezing treatments, and quantifying as the percentage of bud tissue with altered coloration and/or loss of turgor (Obrist et al., 2001). We scored samples as “dead” when (1) the meristematic dome was killed, irrespectively of the extent of the damage of leaf primordia, or (2) the percentage of damaged tissue within the bud exceeded 50%. We also recorded information on the phenological stage of buds (swollen, broken) and on their colouration. Freezing resistance of plant tissue was expressed as lethal temperature 50 (LT₅₀), the estimated temperature at which half of the samples were killed (Sakai and Larcher, 1987). There is generally only a narrow range of temperatures between no damage and complete damage, which allows a relatively precise calculation of this value. We calculated LT₅₀ values using a logistic regression on visual counts of dead and alive buds according to Lim et al. (1998). We calculated LT₅₀ values separately for each clone of each species and plot.

2.5. Non-structural carbohydrate analyses

We determined non-structural carbohydrates (NSC) following a modified method of Wong (1990) as described in detail in Hoch et al., (2002). In short, we extracted about 8 mg of ground plant material in 2 ml of distilled water over steam for 30 minutes. In an

aliquot of the extract, we determined concentrations of the most quantitatively important free sugars, i.e. sucrose, glucose and fructose after enzymatic conversions with invertase and phosphoglucose-isomerase using a glucose hexokinase assay reagent (GHK, Sigma-Aldrich, St. Louis, MO, USA). With the above method, we did not separate the three sugars, but analyzed and reported them as bulk free sugars. Following an enzymatic degradation of starch to free glucose by a crude fungal amylase ('Clarase' from *Aspergillus oryzae*, Enzyme Solutions Pty Ltd., Crydon South, VIC, AUS), we photometrically determined the sum of free sugars plus starch (NSC). The concentration of starch was calculated as NSC minus the free sugars. All concentrations are given as % of dry biomass. All chemicals and enzymes, except the 'Clarase' were purchased from Sigma Diagnostics, St. Louis, MI, USA.

2.6. Calculations and statistical analyses

We evaluated differences in LT₅₀ values across species and snow depths using univariate linear models (LMs) with "Species" or "Snow depth", respectively, as factors, followed by post-hoc Tukey HSD tests. We evaluated the effect of each study species's ecological preferences for different snow depths (see Table 1) on their resistance to freezing (LT₅₀ values) using a Residual Maximum Likelihood (REML) model with "Preference" as a fixed factor and "Species" as a random factor. According to the suggestion in McVicar and Körner (2013), we use °C for temperatures and K for temperature differences throughout this paper to avoid confusion between the two parameters.

We evaluated differences in the soluble sugars, starch and NSC concentrations in the buds of *L. procumbens* and *R. ferrugineum* collected at different snow depths using linear models (LMs), with "Species" and "Snow depth" as fixed factors. We checked residuals were checked for independence, normality and homoscedasticity. We ran LMs, REML and post-hoc tests using JMP 10.0.2 (SAS 9.3 Foundation).

3. Results

3.1. Freezing resistance across species and the relation to phenology and snow depth

The eight Ericaceae species analysed showed remarkable differences in their early spring freezing resistance ($F = 7.6$, d.f. = 7, $P < 0.001$, Fig. 2). The most vulnerable species showed bud freeze injury at a mean of 14 K above the mean frost resistance (LT₅₀) of the most resistant species. The most freezing resistant species was *V. vitis-idaea*, followed by *C. vulgaris* and *L. procumbens*, while the most sensitive species were *V. uliginosum* and *R. ferrugineum* (Fig. 2). Although most species were dormant when collected, 4 % of *V. uliginosum* buds and buds on one of the branches of *V. myrtillus* (which accounted for 0.7 % of the branches studied) were swollen and starting to break.

Species with a preference for microsites with thick snow cover, like *R. ferrugineum*, showed higher LT₅₀ values and, thus, lower resistance to spring freezing than species dominant on ridges and exposed microsites, like *L. procumbens* (Fig. 2 and text below). However, when the ecological preference of species along snow depth gradients was included in REML analyses as a fixed factor, it had no significant effect on the early spring freezing resistance of plants (Fig. 3). This was partly due to the fact that only one species was present in each of the two extreme categories and, therefore, the differences between them were accounted by the random factor "species" rather than the fixed factor "preference". Further, there was a huge variability in the LT₅₀ values of species with a preference for intermediate levels of snow depth. Species with a preference for

microhabitats where snow cover is moderately thin had contrasting frost resistance, like *V. vitis-idaea* and *V. uliginosum*, which showed the minimum and maximum LT₅₀ values registered, respectively (Table 1). Similarly, species dominating microhabitats with moderately thick snow during winter (i.e. class 3 in table 1) included species like *E. carnea* and *C. vulgaris*, with a difference of ca. 8 K in their mean frost resistance.

3.2. Effects of snow depth on the freezing resistance of the buds of *L. procumbens* and *R. ferrugineum*

The two species demonstrated marginally significant differences in their LT₅₀ values across the snow depth gradient ($F = 4.0$, d.f. = 1, $P = 0.059$). However, the effect of snow depth on the freezing resistance of plants was not significant either when both species were analyzed together ($F = 0.5$, d.f. = 4, $P = 0.742$) or separately (Fig. 4). Although samples collected from 1 m and 1.5 m snow depth showed a tendency to be more vulnerable to freezing, particularly in *L. procumbens*, the effect was not significant (Fig. 4).

The analysis of the differences in the carbohydrate content of buds collected at different snow depths indicated that there was a significant effect of snow depth on the sugar content of the buds of both species, with buds collected at shallower snow depths having higher sugar concentrations (Table 2, Fig. 5). *L. procumbens* had significantly higher soluble sugars and NSC concentrations than *R. ferrugineum* in its buds, while concentrations of starch were similar across species (Table 2, Fig. 5). The interaction between “Species” and “Snow depth” was only marginally significant (Table 2), indicating that the trend in the change of the sugar concentration along snow depth gradients was similar across species, irrespective of their different preference for snow cover microsites. In the case of *L. procumbens*, buds collected at 1.5 m had significantly lower sugar concentrations than buds collected at uncovered sites (0 m) (Fig. 5). Similarly, in *R. ferrugineum* buds from 0.5 and 1.5 m snow depth had lower sugar concentrations than buds from 0 m snow depth (Fig. 5). Differences among snow depths in starch or total non-structural carbohydrate concentrations were not significant (Table 2).

4. Discussion

Alpine Ericaceae shrubs showed marked differences in their resistance to early spring freezes, as indicated by the huge variability observed in the LT₅₀ values (i.e. the temperature killing 50% of the buds) in the eight species studied. These differences were not related to variations in snow cover, since all individuals were covered by 1 m of snow, but probably to differences in the phenological stage of plants at the time of sampling and, in some cases, in the ecological preference of species for snow cover microsites. For example, the high vulnerability of *V. uliginosum* to early spring freezes is surprising, since previous studies conducted in other times of the year have shown that this species can be more freezing-resistant than *V. myrtillus* or *R. ferrugineum* (Martin et al., 2010; Taschler and Neuner, 2004; Wheeler et al., 2014). Although the buds of most study species were dormant when collected, some of the *V. uliginosum* buds included in the freezing tests were starting to break, which may explain the high vulnerability to freezing temperatures observed (Fig. 2). Previous studies have revealed how the advance of phenological stages may reduce the freezing resistance of buds (Sakai and Larcher, 1987; Taschler et al., 2004). For example, swollen and breaking buds of *R. ferrugineum* were 4-6 K less freezing-resistant than resting buds (Taschler et al., 2004). Similarly, the ability of many alpine shrubs to promote supercooling by the formation of ice barriers in the buds disappears as development starts in spring (Kuprian

et al., 2014 and references therein). Interestingly, the LT₅₀ values recorded for the buds of *V. uliginosum* in this experiment (-11.1 °C) are similar to those reported for its shoots in the early growing season (Martin et al., 2010). At the other end of our inter-specific comparison, we find *V. vitis-idaea*, an evergreen species that has been reported to have a summer LT₅₀ similar to that of its congener *V. myrtillus* (Stushnoff and Juttala, 1986; Taulavuori et al., 1997; Tolvanen 1997), 19 K warmer than recorded in this study (Taschler and Neuner, 2004). Evergreen woody species from arctic environments are known to delay bud burst as compared to their winter-deciduous neighbours (Shaver and Kummerow, 1992). Indeed, *V. myrtillus* is known to be a non-conservative (early) species (e.g. Rixen et al., 2012). The high freezing resistance of *V. vitis-idaea* in relation to its other two congeners may therefore be due to its relatively late bud burst. This is the first time that the early spring bud freezing resistance has been evaluated for most of the study species. Our results indicate that the relative differences between species were generally consistent with previous studies on the late-spring or summer frost resistance of leaves (Martin et al., 2010; Taschler and Neuner, 2004). For example, *C. vulgaris* showed a relatively high freezing resistance, while *V. myrtillus* and *E. nigrum* showed moderately high LT₅₀ values. The effect of species dominance along snow depth gradients on their early spring freezing was not significant when all species were analysed together. However, the comparison of *R. ferrugineum* and *L. procumbens*, the two species with the largest differences in their snow cover preferences, indicated marginally significant results. In agreement with previous studies of leaf frost hardiness (Taschler and Neuner, 2004), *L. procumbens*, a species with a preference for exposed microsites, showed a tendency to have more freezing-resistant buds in early spring than *R. ferrugineum*, a species typical to snow covered microsites (Fig. 4).

We observed that *V. uliginosum* buds collected under 1m snow depth in the first week of spring were starting to burst, which opens up interesting questions regarding the environmental cues that may have triggered this phenological pattern. Interestingly, breaking buds were not observed in any of the study species collected under 1 m of snow in a previous trial experiment that we carried out one week earlier, which rules out the possibility that bud bursting had started already in autumn prior to snow fall. Solar radiation does not penetrate below 0.50 m snow depth (Starr and Oberbauer, 2003; Woolgrove and Woodin, 1996), so photoperiod cues seem unlikely. Further, subnivean temperatures are kept constant around 0 °C by the insulating layer of snow (Woolgrove and Woodin, 1996), hampering the occurrence of significant aboveground temperature changes. One of the environmental factors that changed in the few days prior to our sampling was the presence of liquid water in the soil as a result of snowmelt and soil thaw (Fig. 1). Together with snow melt, soil thaw has been identified as a key environmental factor triggering the bud burst of plant species in the Arctic (Chapin and Shaver, 1985; Shaver and Kummerow, 1992; Van Wijk et al., 2003), and previous studies indicate *Vaccinium* shrubs can absorb water through stems below snow (Havas, 1971). Our observations further support the role of soil thaw as a potential phenological cue for the alpine sub-shrub *V. uliginosum*.

Buds of *L. procumbens* and *R. ferrugineum* did not differ significantly in their sensitivity to early spring freezing along snow depth gradients. This may partly be due to the large variation across different clones sampled, and the low replication ($N = 3$) in our experiments. Indeed, when we analysed the effect of snow-cover gradients by comparing whole sigmoid curves of the freezing tests instead of just the inflexion point (i.e. the LT₅₀ values), we observed significant differences in the freezing resistance of plants collected at 1 m to 1.5 m snow depth, as compared to those collected 0 and 0.25

m snow depth (Supplementary Materials). Furthermore, plants collected under shallow snow depths were more physiologically hardened than plants from thick snow positions, as indicated by the significantly higher sugar concentrations in the former (Fig. 5). It seems, therefore, that differences in snow cover significantly affect the physiological hardening of plants, with a subsequent (slight) effect on their early spring freezing resistance. The upper 20-50 cm of snow packs are sensitive to diurnal changes in air temperatures (Fierz, 2014; Körner, 2003); and light conditions at snow depth less than 30 cm are sufficient for photosynthesis (Körner, 2003; Starr and Oberbauer, 2003). Thus, plants from uncovered microsites or covered by less than 50 cm of snow were more exposed to winter conditions in the weeks prior to sampling, and consequently more physiologically hardened, than individuals sheltered by 1 m snow or more. Interestingly, our sugar concentration data suggest that the few warm days recorded prior to the sampling date and the start of snow melt and soil thawing were not sufficient to de-harden individuals of *L. procumbens* and *R. ferrugineum* growing in exposed microsites.

5. Conclusions

Our results indicate that alpine Ericaceae shrubs show a high inter-species variability in the bud freezing resistance during the first weeks of spring. The observed differences in freezing vulnerability could be related to differences in the bud phenology of species and their preference for snow cover microsites. Some species, like *V. uliginosum*, were found to burst their buds under 1 m of snow, which reduces the likelihood of dependence on cues like air temperatures or the photoperiod, and suggest a potential role of snow melting and the presence of running water in the soil. Differences in snow cover significantly affect the physiological hardening of plants in early spring. Individuals growing in more exposed microsites are hardier and show moderately higher freezing resistance than individuals from snow banks.

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Author contributions

SP, CR, SW designed the study; SP, CR and SW did the fieldwork; AL designed and ran the freezing tests; all authors evaluated freezing damage; GH ran the carbohydrate analyses; SP and AL statistically analyzed the data; all authors interpreted the results, drafted the article and revised it critically. All authors approved the final version of the manuscript. SP (s.palacio@ipe.csic.es), AL (armando.lenz@unibas.ch), SW (sonja.wipf@wsl.ch) and CR (rixen@slf.ch) take responsibility for the integrity of the work as a whole, from inception to finished article.

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Figure caption

Fig. 1 Air temperatures in the study site between the 1st January 2012 and the 30th June 2012. Mean (solid line) and minimum and maximum (dotted lines) daily temperatures are shown. The sampling date is indicated by a vertical broken line.

Fig. 2 LT₅₀ values for the buds of eight different species collected under 1 m of snow cover in the Swiss Alps on the 2nd May 2012. Average values across replicates are shown $\pm$ SE ($N = 3$). Different letters indicate significant differences among species after HSD Tukey post-hoc tests ($\alpha = 0.05$).

Fig. 3 LT₅₀ values for the buds of species demonstrating different categories of ecological preference for microsites along snow depth gradients. Categories in X axis include: 1 = exposed ridges or lightly snow covered areas, 2 = light to moderately snow covered areas, 3 = moderately to thickly snow covered areas, 4 = snow banks and thickly snow covered areas. Average values across replicates are shown $\pm$ SE ($N = 3$ in categories 1 and 4, $N = 9$ in categories 2 and 3). Differences between categories were not significant (see F-ratio and P-value in the inlet) after a REML model with “preference” as a fixed factor and “species” as a random factor ($\alpha = 0.05$).

Fig. 4. LT₅₀ values for the buds of *L. procumbens* (filled circles) and *R. ferrugineum* (empty circles) collected under different snow depths. Average values across replicates are shown $\pm$ SE ($N = 3$). Within species, the effect of snow depth on the freezing resistance of buds was not significant after an ANOVA test with “Snow depth” as an ordinal factor: $F = 0.5$, d.f. = 4, $P = 0.708$ and $F = 0.3$, d.f. = 4, $P = 0.900$ for *L. procumbens* and *R. ferrugineum*, respectively ($\alpha = 0.05$).

Fig. 5. Soluble sugar (SS) concentrations of buds of *L. procumbens* and *R. ferrugineum* collected at different snow depths. Values are means $\pm$ SE (error bars), $N = 3$. Different letters indicate significant differences within each species among snow depths after HSD Tukey post-hoc tests ($\alpha = 0.05$).

Table 1. Ecological preference of study species along snow depth gradients and their altitudinal distribution limit in the study area.

Species‡	Preference for snow depth microsites*†	Altitudinal limit¥
<i>Loiseleuria procumbens</i> (L.) Desv.	1	2780

<i>Empetrum nigrum</i> ssp.	2	
<i>hermaphroditum</i> (Hagerup) Böcher		2700
<i>Vaccinium uliginosum</i> L.	2	2760
<i>Vaccinium vitis-idaea</i> L.	2	2934
<i>Calluna vulgaris</i> (L.) Hull	3	2620
<i>Erica carnea</i> L.	3	2550
<i>Vaccinium myrtillus</i> L.	3	2820
<i>Rhododendron ferrugineum</i> L.	4	2624

‡ Species names follow Flora Helvetica (Lauber and Wagner 2007).

* 1 = exposed ridges or lightly snow covered areas, 2 = light to moderately snow covered areas, 3 = moderately to thickly snow covered areas, 4 = snow banks and thickly snow covered areas

† Source (Ellenberg 1988; Körner 2003; Rixen et al., 2010), and own field observations)

¥ Source (Braun-Blanquet and Rübel, 1936).

Table 2. Summary statistics for LM models exploring the effect of “Species” and “Snow depth” on the soluble sugars, starch and total non-structural carbohydrates concentrations of the buds of *L. procumbens* and *R. ferrugineum*. Significant effects are highlighted in bold type.

Response variable	Factors	F-ratio	d.f.	P-value
Soluble sugars	<i>Species</i>	95.2	1	<.001
	<i>Snow depth</i>	6.6	4	0.002
	<i>Species*Snow depth</i>	2.8	4	0.054
Starch	<i>Species</i>	0.3	1	0.585
	<i>Snow depth</i>	1.5	4	0.246
	<i>Species*Snow depth</i>	2.1	4	0.118
NSC	<i>Species</i>	72.8	1	<.001
	<i>Snow depth</i>	0.9	4	0.509
	<i>Species*Snow depth</i>	0.8	4	0.519

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5