

Evergreen alpine shrubs have high freezing resistance in spring, irrespective of snowmelt timing and exposure to frost: an investigation from the Snowy Mountains, Australia

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Abstract Over winter, alpine plants are protected from low-temperature extremes by a blanket of snow. Climate change predictions indicate an overall reduction in snowpack and an earlier thaw; a situation which could expose the tips of shrubs which extend above the snowpack to freezing events in early spring, and cause foliar frost damage during the onset of physiological activity. We assessed the photosynthetic responses of freezing-damaged shrub leaves from an assay of freezing temperatures in the Snowy Mountains in south-eastern Australia, using chlorophyll fluorometry *ex situ*. We sampled leaves that were exposed early during the spring thaw and leaves that were buried in snow for up to two extra weeks, from four evergreen shrub species at monthly intervals following the period of snowmelt. Freezing resistance (estimated from LT_{50}) was poorest at the earliest spring sampling

time, in both exposed above-snow and protected below-snow foliage in all species. Protected foliage in early spring had lower freezing resistance than exposed foliage, but not significantly so. By the third sampling time, freezing resistance was significantly better in the lower protected foliage (LT_{50} of -14) compared with the upper exposed foliage (LT_{50} of -10) in one species. Over the course of spring, freezing resistance improved significantly in all species, with LT_{50} values of between -10 and -15 °C by the third sampling time, which is lower than the minimum air temperatures recorded at that time (> -5 °C). The results indicate that the dominant evergreen shrub species in this area may only be susceptible to freezing events very early in spring, before a period of frost-hardening occurs after snowmelt. Later in spring, these alpine shrubs appear frost hardy, thus further perpetuating the positive feedbacks surrounding shrub expansion in alpine areas.

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Introduction

In an alpine landscape with a mosaic of plant communities, shrubs tend to be absent from areas that have long-lasting snow cover and short-growing

seasons (such as snowbanks and lee-side ridges), and rarely grow in exposed snow-free areas. Shrubs are more common in protected and sunny positions. However, repeat photography, long-term monitoring, field warming experiments and dendrochronology have revealed that alpine and arctic tundra shrubs are responding to the effects of global warming, including rising temperatures and declining snowpack, by encroaching into alpine herbfields and grasslands where they were previously absent (Myers-Smith et al. 2011, 2015).

Despite earlier snowmelt across parts of the alpine tundra, some shrubs can prolong winter processes; they can modify local snow accumulation patterns by trapping drifting snow, leading to increases in snowpack and insulating soils. This protective layer of snow may promote a second feedback, whereby the deeper snowpack and warmer, more moist soils combine with shrub leaf litter to increase microbial activity (Buckeridge and Grogan 2008; Buckeridge et al. 2010; Larsen et al. 2007; Warren and Taranto 2011), which in turn, boosts soil nutrient cycling and ultimately promotes shrub growth and expansion. As snowpack declines in future decades, it is unclear how important the timing of snowfall, snowmelt and exposure to very low temperatures will be for these interacting processes.

While photosynthesis can occur under snow at temperatures down to -6°C at reduced rates (approximately 7% transmittance of net radiation at 20 cm depth of snow) (Körner 2003), snowmelt hails the beginning of the growing season in earnest, when plants begin to rapidly photosynthesize at peak levels. At the time of snowmelt, evergreen shrubs are likely subjected to freezing temperatures and overnight frosts, because their upper branches are generally exposed first as they spring-up upon being released from snow and then protrude from the snowpack, while the rest of the plant and other species remain buried and protected under the snow. Given the regular early exposure of the upper foliage of some shrub species, these shrubs may therefore be generally frost hardy (Bannister 2007; Neuner 2014), despite the risk of freezing damage to newly developing tissues and buds (Inouye 2000). If snowmelt occurs much earlier than usual, as is predicted under a warming climate (Hennessy et al. 2007), most plants will not have acclimated (hardened) and may be exposed to spring frosts and be especially vulnerable to freezing damage at a time when their photosystems are most sensitive to

low-temperature extremes, and when new tissues and buds are highly sensitive to very low temperatures (Inouye 2000; Inouye et al. 2002; Palacio et al. 2015; Wheeler et al. 2014). Through acting on developing tissues, these processes of freezing damage may even reduce overall carbon fixation and growth opportunities of alpine shrubs in early spring. Resistance to and avoidance of freezing in alpine shrubs may be offered by sclerophyllous leaves (particularly in Australia), landscape position, snowmelt timing and/or an inherent resistance provided by supercooling and internal ice partitioning (Briceño et al. 2014; Neuner 2014; Venn et al. 2013). Sclerophyllous evergreen alpine shrubs are notoriously freezing resistant; some species can withstand temperatures down to -18°C (Bannister 2007; Venn et al. 2013). The period of frost-hardening (acclimation to very low temperatures) varies between species (Bannister 2007; Bannister et al. 2005), while very few are expected to show maximum resistance to freezing immediately after they are released from snow, given the constant temperatures under snow (Inouye and McGuire 1991).

Here, we investigate the differences in freezing resistance between alpine shrub foliage that is released from snow early, compared to foliage that is protected and released up to two weeks later; with the view to linking shrub expansion, declining snowpack and the freezing resistance of alpine shrubs. Specifically we ask the following: (1) Does the timing of snow release affect shrub foliage freezing resistance in early spring? (2) Are there patterns in freezing resistance with respect to the timing of exposure across shrub species? (3) Does freezing resistance improve through early spring among species?

Methods

Study site and shrub species

The study was conducted at 1900 m a.s.l. ± 10 m in a closed heath community on a north-west facing slope near Charlotte Pass in the Snowy Mountains, south-eastern Australia. Annual precipitation in this region is in the range of 1800–3100 mm per year with about 60% of this falling snow mainly between June and September (Costin et al. 2000). Peak snow depth varies with landscape position and aspect, but is generally 1.3 m on the north-facing slope near

Charlotte Pass (Green K *unpublished data*). Snowmelt across the study site is relatively even, with no landscape features such as large rocks or abrupt topographic features affecting the regular pattern of snowmelt. Foliar freezing resistance was assessed for four common representative alpine shrub species of varying height that are found throughout the region (Table 1).

Foliage sampling

Leaf material was collected during the period of the spring thaw in September, October and November 2010 from four randomly chosen replicate individuals of the four species (16 target shrubs in total). All the target shrubs were within 5–10 m of each other and spread across the slope in a band of the same altitude and aspect of approximately 10 × 40 m. The target shrubs grow in relatively even abundance across the site and do not clump together. Other shrubs as well as graminoid and herbaceous species were also present at the site. The target shrubs were tagged at the first sampling time in order to revisit those individuals at subsequent sampling times. At the first sampling time (9th September 2010), foliage samples of fully expanded, healthy mature leaves (from the previous season) were collected from the four randomly chosen replicate shrubs of each species that could be identified by their stems protruding from the snowpack. Foliage was collected from those exposed stems above the snow, and from protected stems by digging down through the snowpack that was still buried in snow at that time. By the second collection (18th October), the snow had completely melted at the site, and hence during the second and third (15th November) collection times, foliage was taken from the upper branches that would have been exposed early, and from lower branches that would have been exposed later

(hereafter referred to ‘exposed’ and ‘protected’ foliage). At each collection time, 10 shoots with leaves attached (five from the exposed upper foliage and five from protected lower foliage to go into the freezing assay of different temperatures) were harvested from each target individual from each species, kept damp in sealed polyethylene bags and transported to the laboratory in a pre-chilled insulated container. Air temperatures were recorded bi-hourly by two shaded Tinytag plus dataloggers attached to the upper and lower branches of one of the target shrubs. Snowmelt date was determined retrospectively from fluctuations in the temperature trace of dataloggers, as temperatures under snow remain stable between 0 and 1 °C.

Assessing foliar freezing resistance

We measured foliar freezing resistance by subjecting the sampled leaves to a freezing assay, and then using the ratio between variable and maximum chlorophyll fluorescence (the effective quantum yield, F_v/F_m ratio) to measure damage to photosystem 2 (PSII) as per the methods described in Bannister (2005), Bannister et al. (2005), Bannister and Lord (2006), Bannister (2007) and Venn et al. (2013). This method uses the temperature that causes a 50% reduction in initial F_v/F_m (FT₅₀; objectively measured using a chlorophyll fluorometer), as has previously been compared with subjective visual estimates of 50% damage to leaves (LT₅₀) (Cunningham and Read 2006). Overall, studies comparing LT₅₀ with FT₅₀ have revealed differences in leaf damage of less than ± 1 °C of damaging temperatures (Bannister 2007). Values of FT₅₀ have been shown to correlate well with those of LT₅₀ derived from electrolyte leakage techniques (Raymond et al. 1992; Lindgren and Hållgren 1993); however, chlorophyll fluorometry techniques are better suited to the sclerophyllous leaves of Australian alpine shrubs (Bannister 2005), as hard tissues may not release electrolytes readily and may give false estimates of high freezing resistance (Bannister 2007; Reitsma 1994).

Upon arrival at the laboratory (approximately 1 h after leaf collection), plant material was transferred to a dark cooling pre-treatment of 4 °C for 8 h to allow for uniform cold acclimatization across samples and to simulate the length of a night before an early morning frost, according to the protocols in Bannister (2005)

Table 1 The study species including their height (m) at the study site

Species	Family	Height (m)
<i>Grevillea australis</i>	Proteaceae	0.8
<i>Nematolepis ovatifolia</i>	Rutaceae	1.2
<i>Prostanthera cuneata</i>	Lamiaceae	1.2
<i>Leucopogon montanus</i>	Ericaceae	0.6

and Bannister and Lord (2006). During this pre-treatment, samples were wrapped in damp paper towels and in sealed plastic bags to ensure full hydration, as low leaf water potentials can increase the likelihood of supercooling and seeding of extracellular ice nucleation (Goldstein et al. 1985; Kuprian et al. 2014; Wisniewski et al. 2002). Following the pre-treatment, and while still wrapped in damp paper towel and sealed in plastic bags, the foliage samples (with leaves still attached to their stems) from the four replicate plants of each species were subjected to one of four freezing treatments in the assay (-3 , -10 , -15 , -22 and 4 °C control) for 8 h using thermostatically controlled freezers. The samples were placed directly into the target temperature treatments; however, the damp towel and sealed plastic bags provided sufficient insulation so that cooling to the target temperature occurred at a rate of ca. 7–8 °C per hour across samples (as determined by wrapping iButton temperature loggers (Thermochron DS1922L-F5) in the same manner and subsequently examining the temperature data). The target temperatures encompass the range of freezing temperatures recorded for the region and are comparable with New Zealand, Australian and Chilean alpine plant freezing tolerance studies (Bannister 2007; Bannister et al. 2005; Sierra-Almeida et al. 2009; Venn et al. 2013). This method of direct cooling thereby measures the current freezing resistance rather than the hardening capacity of the plant material (Larcher et al. 2010). Control samples were likewise hydrated and held in sealed plastic bags at 4 °C.

After freezing, leaves were wrapped in moist cloths and sealed into plastic bags in the dark at room temperature (15–20 °C). After 3 days, freezing damage to PSII was assessed using a PAM chlorophyll fluorometer (Walz Effeltrich, Germany) (Venn et al. 2013) to determine the ratio of variable to maximum fluorescence of the sample (F_v/F_m) of dark-adapted leaves. Lethal temperature (LT_{50} , measured as causing 50% damage to the photosynthetic apparatus) was determined by interpolating the temperature of the highest F_v/F_m under 50% F_m and the lowest F_v/F_m over 50% F_m (Bannister et al. 2005). The F_v/F_m of control samples (which usually showed the highest ratio of F_v/F_m , typically between 0.7 and 0.8) also provided an estimate of PSII efficiency during the *ex situ* assay procedure. The LT_{50} values here reflect the temperature at which PSII functioning decreased to

50% relative to the controls, and we infer freezing resistance from this temperature.

Any statistical differences in the mean LT_{50} values between exposed/upper foliage and protected/below-snow foliage within each species were determined by examining the overlap of 95% confidence intervals of the mean (Cumming and Finch 2005). The relative change in LT_{50} for each species through early spring was analysed by repeated measures ANOVA. In cases where the sphericity assumption was violated, we used the more conservative Greenhouse–Geisser epsilon corrected P value (Quinn and Keough 2003). We also used simple linear regression to look for significant trends in LT_{50} values with sampling time for each species through early spring. Statistical analyses were performed in SYSTAT version 10 (SPSS Inc. 2000).

Results

Snowmelt at the study site occurred 12 days after the first foliage samples were taken in September. Over the course of the study (9 September to 15 November, 67 days), temperatures below 0 °C were recorded on 43 days in the exposed upper foliage, and on 49 days in the lower protected branches that were initially buried in snow. The buried foliage had stable temperatures hovering around 0 °C for almost two weeks longer than the exposed upper foliage, which was exposed to minimum air temperatures between 0 and -7 °C (Fig. 1). Maximum daily temperatures over the study period amongst the exposed upper shrub foliage ranged between -1 and 18 °C, and daily minimum temperatures ranged between -9 and 11 °C (Fig. 1). Maximum daily temperatures recorded from the lower protected branches ranged between -3 and 18 °C, and minimum daily temperatures ranged between -10 and 12 °C.

Springtime freezing resistance varied between the four shrub species, with mean LT_{50} values ranging from -6 to -18 °C over the course of the study (Fig. 2). The timing of snow release did not overwhelmingly cause significant differences in freezing resistance throughout the course of the study. At the onset of snowmelt in September, the upper exposed foliage did appear to have poor freezing resistance (higher LT_{50}) in *G. australis*, *N. ovatifolia* and *P. cuneata*, but it was not significantly different to the lower, protected foliage. However, after examining

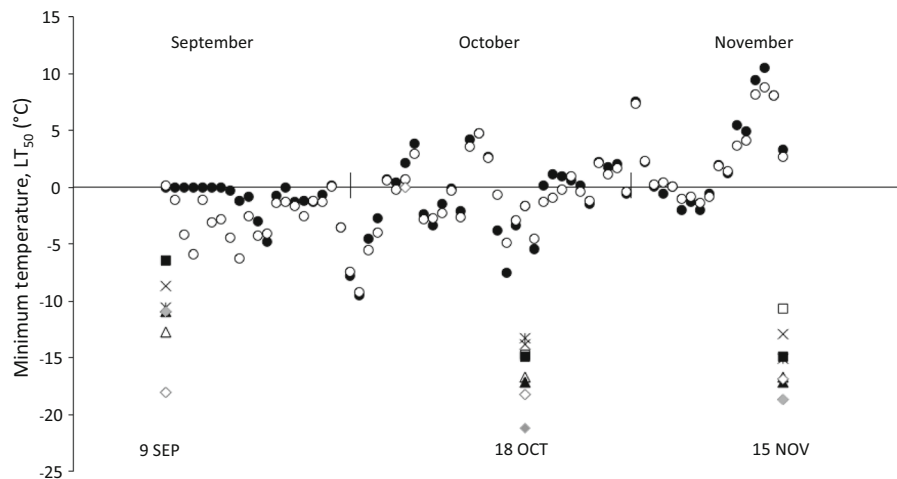


Fig. 1 The minimum air temperatures at the study site over the course of early spring recorded from within the lower protected branches of the target shrubs (closed circles) and at the exposed canopy (shaded) branches of the target shrubs (open circles), and the LT_{50} values of the target shrubs measured at three sample times over early spring (9 September, 18 October and 15

November 2010), for lower protected foliage (closed shapes) and upper exposed foliage (open shapes) in *Grevillea australis* (black triangles), *Nematolepis ovatifolia* (black squares), *Prostanthera cuneata* (grey diamonds) and *Leucopogon montanus* (black crosses)

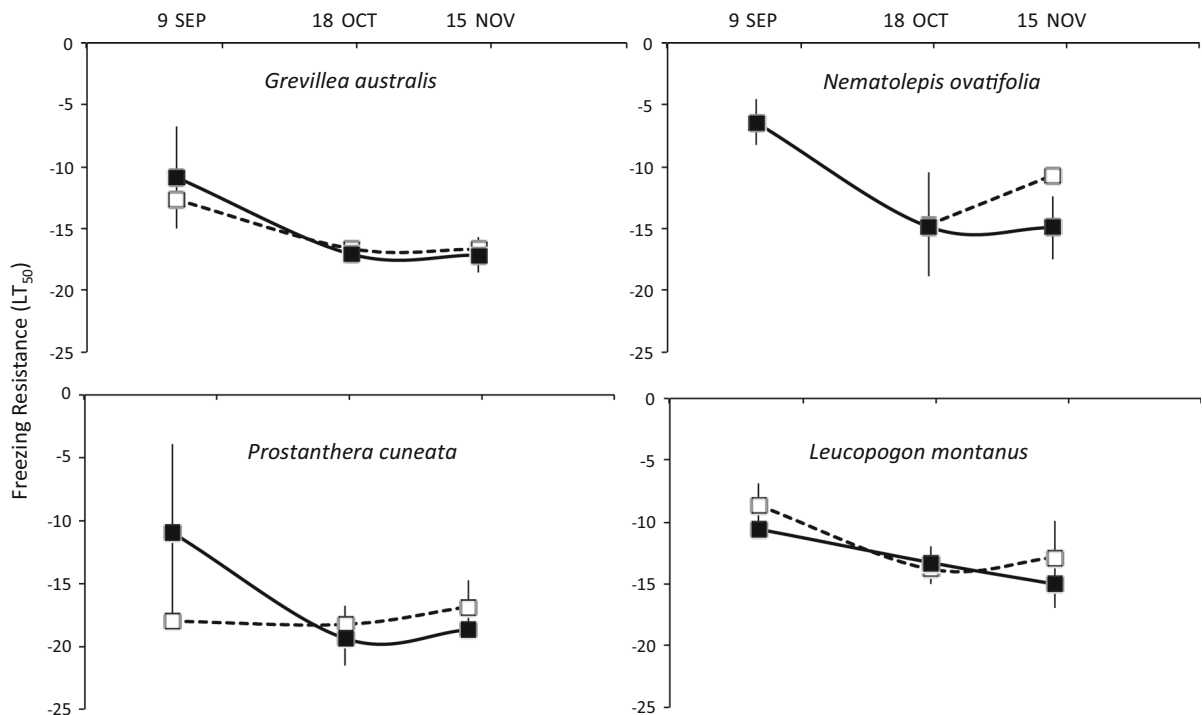


Fig. 2 Mean (\pm 95% confidence intervals) foliar freezing resistance, inferred from LT_{50} for four shrub species sampled during the spring thaw; protected lower foliage (closed squares and dark lines), exposed upper foliage (open squares and dashed lines)

the overlap of confidence intervals around the mean LT_{50} values, the lower, protected foliage of *N.*

ovatifolia did show significantly better freezing resistance (lower LT_{50}) at the third sampling time in

November, although this pattern was not replicated in the other three species (Fig. 2).

Repeated measures ANOVAs revealed a significant overall effect of the sampling time on freezing resistance (LT_{50}), ($F = 18.53$, $P < 0.001$), although there were no significant interactions between the sampling time and samples from above or below the snow, nor with different species. There was also a significant effect of species on freezing resistance (LT_{50}) ($F = 6.81$, $P = 0.014$), but no significant effect of sampling above or below the snow, nor was there a significant interaction between these two factors. For all species, freezing resistance generally improved (LT_{50} values decreased) over the sampling period, with the highest resistance to freezing occurring during the October sampling time. In all species and exposed or protected foliage, simple linear regressions using the raw LT_{50} data also revealed significant trends with freezing resistance improving over the course of early spring (Table 2).

Discussion

The alpine shrubs studied here are most at risk of frost damage immediately after snowmelt and during spring (September and October), when their freezing resistance is poorest. The earlier exposure of upper foliage to frost did not, however, result in poor freezing resistance later in spring, as all shrub species appeared to frost-harden sufficiently over the course of the study and the recorded minimum temperatures were

generally higher than the estimated freezing resistance (LT_{50} values) of the shrubs.

The timing of snowmelt, and hence the timing of the exposed and protected foliage, only significantly affected the foliar freezing resistance in *N. ovatifolia*, where the protected lower foliage had significantly better freezing resistance than the upper exposed foliage at the third sampling time, when all leaves would have been frost-hardened. Overall, the freezing resistance of the alpine shrubs tested here is sufficient to survive known minimum temperatures and therefore these species are unlikely to be detrimentally affected by even the most severe frosts. The only exception is *N. ovatifolia* in early spring during snowmelt, which had the poorest freezing resistance in the lower, protected foliage ($LT_{50} - 7^{\circ}\text{C}$).

The lowest air temperature recorded over the period of the study was -9°C ; however, the lowest recorded temperature of the region is -23°C , recorded at the cold air drainage basin at Charlotte Pass village (1760 m) in winter on the 29 June 1994 (Bureau of Meteorology 2017), 1.8 km from the study site. Severe frost impacts on vegetation were not reported in 1994, as most plants would have been protected under snow at that time. The lowest springtime (September to November) temperatures for Charlotte Pass village range between -16 and -9°C . Thus, all four shrub species considered here, which have exposed foliage LT_{50} values between -6 and -13°C during the onset of spring, may be at risk of freezing damage if air temperatures drop below -15°C and they have already been released snow.

Overall, the foliar freezing resistance values of the shrubs studied here are within the range of LT_{50} values reported for alpine shrubs in similar environments during the onset of spring and throughout the growing season in New Zealand (Bannister 2007; Bannister and Lord 2006), the Rocky Mountains, Colorado, USA (Loik et al. 2004), in the Austrian Alps (Taschler and Neuner 2004), across the Swiss Alps (Martin et al. 2010; Palacio et al. 2015; Wheeler et al. 2014), the central Chilean Andes (Sierra-Almeida et al. 2009) and south-eastern Australia (Venn et al. 2013). Researchers seeking predictable correlative patterns in shrub freezing resistance across elevation gradients (Loik and Redar 2003; Venn et al. 2013; Wheeler et al. 2014), growing season, snowmelt and snow-depth gradients (Palacio et al. 2015; Sakai and Larcher 2012; Sierra-Almeida et al. 2009; Venn et al. 2013; Wipf

Table 2 The results of simple linear regressions between species LT_{50} values and sampling date from exposed and protected foliage

Species/foliage	R^2	P value
<i>Grevillea australis</i> /exposed	0.87	0.001
<i>Grevillea australis</i> /protected	0.83	0.001
<i>Nematolepis ovatifolia</i> /exposed	0.81	< 0.001
<i>Nematolepis ovatifolia</i> /protected	0.81	< 0.001
<i>Prostanthera cuneata</i> /exposed	0.81	0.002
<i>Prostanthera cuneata</i> /protected	0.82	< 0.001
<i>Leucopogon montanus</i> /exposed	0.82	< 0.001
<i>Leucopogon montanus</i> /protected	0.77	< 0.001

et al. 2009) and under experimental warming, imposed drought and CO₂ additions (Martin et al. 2010; Rixen et al. 2012; Sierra-Almeida et al. 2016), mostly report species-specific responses rather than general observations in ecological freezing resistance in alpine shrubs (Neuner 2014). However, in most alpine regions, shrubs have a low overall risk of freezing damage in their respective environments, while early spring frost damage remains a potential risk (Neuner 2014; Wheeler et al. 2014).

There is substantial evidence of common shrubs, including the species investigated here, increasing in abundance in the Snowy Mountains and in other alpine regions of south-eastern Australia, particularly in habitats such as snowpatches, in which shrubs were previously excluded (McDougall 2003; Pickering et al. 2014; Scherrer and Pickering 2005; Venn et al. 2011, 2012, 2014; Williams et al. 2015). Historically, the coldest parts of the Snowy Mountains landscape, the lower-elevation grassy valleys and cold air drainage basins with marginal snow cover, have remained free of woody plants. Despite the overall frost hardiness of shrubs, cold temperature extremes during the vulnerable growth stages will likely continue to limit shrub distribution in these positions, and prevent shrub encroachment in such cold air drainage areas and on the highest, exposed ridgelines (Neuner 2014; Sierra-Almeida and Cavieres 2012), despite decadal warming trends in air temperature both globally and locally (Martin et al. 2010; Sánchez-Bayo and Green 2013). Given the high freezing resistance of the common alpine shrubs tested here, it is unlikely that future frost events will limit the spread of these species, except for into these very cold landscape positions where shrubs are currently excluded.

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References

- Bannister P (2005) Frost resistance of the New Zealand narrow-leaved snow tussock grass, *Chionochloa rigida*. *NZ J Bot* 43:425–430
- Bannister P (2007) Godley review: a touch of frost? Cold hardiness of plants in the southern hemisphere. *NZ J Bot* 45:1–33
- Bannister P, Lord JM (2006) Comparative winter frost resistance of plant species from southern Africa, Australia, New Zealand, and South America grown in a common environment (Dunedin, New Zealand). *NZ J Bot* 44:109–119
- Bannister P, Maegli T, Dickinson KJM, Halloy SRP, Knight A, Lord JM, Mark AF, Spenser KL (2005) Will loss of snow cover during climatic warming expose New Zealand alpine plants to increased frost damage? *Oecologia* 144:245–256
- Briceño VF, Harris-Pascal D, Nicotra AB, Williams E, Ball MC (2014) Variation in snow cover drives differences in frost resistance in seedlings of the alpine herb *Aciphylla glacialis*. *Environ Exp Bot* 106:174–181
- Buckeridge KM, Grogan P (2008) Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. *Appl Soil Ecol* 39:210–222
- Buckeridge KM, Zufelt E, Chu H, Grogan P (2010) Soil nitrogen cycling rates in low arctic shrub tundra are enhanced by litter feedbacks. *Plant Soil* 330:407–421
- Costin AB, Gray M, Totterdell CJ, Wimbush DJ (2000) *Kosciuszko Alpine Flora*. CSIRO, Melbourne
- Cumming G, Finch S (2005) Inference by eye—confidence intervals and how to read pictures of data. *Am Psychol* 60:170–180
- Cunningham S, Read J (2006) Foliar temperature tolerance of temperate and tropical evergreen rain forest trees of Australia. *Tree Physiol* 26:1435–1443
- Goldstein G, Rada F, Azocar A (1985) Cold hardiness and supercooling along an altitudinal gradient in Andean giant rosette species. *Oecologia* 68:147–152
- Hennessy K, Whetton P, Walsh K, Smith I, Bathols J, Hutchinson M, Sharples J (2007) Climate change effects on snow conditions in mainland Australia and adaptation at ski resorts through snowmaking. *Climate Res* 35:255
- Inouye DW (2000) The ecological and evolutionary significance of frost in the context of climate change. *Ecol Lett* 3:457–463
- Inouye DW, McGuire AD (1991) Effects of snowpack on timing and abundance of flowering in *Delphinium nelsonii* (Ranunculaceae): implications of climate change. *Am J Bot* 78:997–1001
- Inouye DW, Morales MA, Dodge GJ (2002) Variation in timing and abundance of flowering by *Delphinium barbeyi* Huth (Ranunculaceae): the roles of snowpack, frost, and La Nina, in the context of climate change. *Oecologia* 130:543–550
- Körner C (2003) *Alpine plant life: functional plant ecology of high mountain ecosystems*. Springer, Berlin
- Kuprian E, Briceño VF, Wagner J, Neuner G (2014) Ice barriers promote supercooling and prevent frost injury in reproductive buds, flowers and fruits of alpine dwarf shrubs throughout the summer. *Environ Exp Bot* 106:4–12

- Larcher W, Kainmüller C, Wagner J (2010) Survival types of high mountain plants under extreme temperatures. *Flora-Morphol Distrib Funct Ecol Plan* 205(1):3–18
- Larsen KS, Grogan P, Jonasson S, Michelsen A (2007) Respiration and microbial dynamics in two subarctic ecosystems during winter and spring thaw: effects of increased snow depth. *Arct Antarct Alp Res* 39:268–276
- Lindgren K, Hällgren J-E (1993) Cold acclimation of *Pinus contorta* and *Pinus sylvestris* assessed by chlorophyll fluorescence. *Tree Physiol* 13:97–106
- Loik ME, Redar SP (2003) Microclimate, freezing tolerance, and cold acclimation along an elevation gradient for seedlings of the Great Basin Desert shrub, *Artemisia tridentata*. *J Arid Environ* 54:769–782
- Loik ME, Still CJ, Huxman TE, Harte J (2004) In situ photosynthetic freezing tolerance for plants exposed to a global warming manipulation in the Rocky Mountains, Colorado, USA. *New Phytol* 162:331–341
- Martin M, Gavazov K, Körner C, Hättenschwiler S, Rixen C (2010) Reduced early growing season freezing resistance in alpine treeline plants under elevated atmospheric CO₂. *Glob Change Biol* 16:1057–1070
- McDougall KL (2003) Aerial photographic interpretation of vegetation changes on the Bogong High Plains, Victoria, between 1936 and 1980. *Aust J Bot* 51:251–256
- Myers-Smith IH, Forbes BC, Wilmking M, Hallinger M, Lantz T, Blok D, Tape KD, Macias-Fauria M, Sass-Klaassen U, Lévesque E, Boudreau S, Ropars P, Hermanutz L, Trant A, Siegwart Collier L, Weijers S, Rozema J, Rayback SA, Schmidt NM, Schaepman-Strub G, Wipf S, Rixen C, Ménard CB, Venn S, Goetz S, Andreu-Hayles LA, Elmendorf S, Ravolainen V, Welker J, Grogan P, Epstein HE, Hik DS (2011) Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. *Environ Res Lett* 6(4):045509
- Myers-Smith IH, Elmendorf SC, Beck PS, Wilmking M, Hallinger M, Blok D, Tape KD, Rayback SA, Macias-Fauria M, Forbes BC (2015) Climate sensitivity of shrub growth across the tundra biome. *Nat Clim Change* 5:887–891
- Neuner G (2014) Frost resistance in alpine woody plants. *Front Plant Sci* 5:654
- Palacio S, Lenz A, Wipf S, Hoch G, Rixen C (2015) Bud freezing resistance in alpine shrubs across snow depth gradients. *Environ Exp Bot* 118:95–101
- Pickering C, Green K, Barros AA, Venn S (2014) A resurvey of late-lying snowpatches reveals changes in both species and functional composition across snowmelt zones. *Alp Bot* 124:93–103
- Quinn GP, Keough MJ (2003) Experimental design and data analysis for biologists. Cambridge University Press, Port Melbourne
- Raymond CA, Owen CV, Harwood CE (1992) Screening eucalypts for frost resistance in breeding programs. *Can J For Sci* 22:1271–1277
- Reitsma L (1994) The frost resistance of some native plants from the Central Volcanic Plateau, North Island, New Zealand, in relation to plant succession. *NZ J Bot* 32:217–226
- Rixen C, Dawes MA, Wipf S, Hagedorn F (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO₂ enrichment and soil warming. *Oikos* 121:1532–1543
- Sakai A, Larcher W (2012) Frost survival of plants: responses and adaptation to freezing stress. Springer Science & Business Media, New York
- Sánchez-Bayo F, Green K (2013) Australian snowpack disappearing under the influence of global warming and solar activity. *Arct Antarct Alp Res* 45:107–118
- Scherrer P, Pickering CM (2005) Recovery of alpine vegetation from grazing and drought: data from long-term photo-quadrats in Kosciuszko National Park, Australia. *Arct Antarct Alp Res* 37:574–584
- Sierra-Almeida A, Cavieres LA (2012) Summer freezing resistance of high-elevation plant species changes with ontogeny. *Environ Exp Bot* 80:10–15
- Sierra-Almeida A, Cavieres LA, Bravo LA (2009) Freezing resistance varies within the growing season and with elevation in high-Andean species of central Chile. *New Phytol* 182:461–469
- Sierra-Almeida A, Reyes-Bahamonde C, Cavieres LA (2016) Drought increases the freezing resistance of high-elevation plants of the Central Chilean Andes. *Oecologia* 181:1011–1023
- Taschler D, Neuner G (2004) Summer frost resistance and freezing patterns measured in situ in leaves of major alpine plant growth forms in relation to their upper distribution boundary. *Plant, Cell Environ* 27:737–746
- Venn SE, Green K, Pickering CM, Morgan JM (2011) Using plant functional traits to explain community composition across a strong environmental filter in Australian alpine snowpatches. *Plant Ecol* 212:1491–1499
- Venn SE, Pickering CM, Green K (2012) Short-term variation in species richness across an altitudinal gradient of alpine summits. *Biodivers Conserv* 21:3157–3186
- Venn SE, Morgan JW, Lord JM (2013) Foliar freezing resistance of Australian alpine plants over the growing season. *Austral Ecol* 38:152–161
- Venn S, Pickering C, Green K (2014) Spatial and temporal functional changes in alpine summit vegetation are driven by increases in shrubs and graminoids. *AoB Plants* 6:plu008
- Warren CR, Taranto MT (2011) Ecosystem respiration in a seasonally snow-covered subalpine grassland. *Arct Antarct Alp Res* 43:137–146
- Wheeler J, Hoch G, Cortés AJ, Sedlacek J, Wipf S, Rixen C (2014) Increased spring freezing vulnerability for alpine shrubs under early snowmelt. *Oecologia* 175:219–229
- Williams R, Wahren CH, Stott K, Camac J, White M, Burns E, Harris S, Nash M, Morgan J, Venn S (2015) An International Union for the Conservation of Nature Red List ecosystems risk assessment for alpine snow patch herbfields, South-Eastern Australia. *Austral Ecol* 40:433–443
- Wipf S, Stoeckli V, Bebi P (2009) Winter climate change in alpine tundra: plant responses to changes in snow depth and snowmelt timing. *Climatic Change* 94:105–121
- Wisniewski M, Fuller M, Glenn DM, Gusta L, Duman J, Griffith M (2002) Extrinsic ice nucleation in plants. In *Plant cold hardiness*. Springer, New York, pp. 211–221