

High tolerance of a high-arctic willow and graminoid to simulated ice encasement

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Climate change-induced snow thaw and subsequent accumulation of ice on the ground is a potential, major threat to snow-dominated ecosystems. While impacts of ground-ice on arctic wildlife are well explored, the impacts on tundra vegetation is far from understood. We therefore tested the vulnerability of two high-arctic plants, the prostrate shrub *Salix polaris* and the graminoid *Luzula confusa*, to ice encasement for 60 days under full environmental control. Both species were tolerant, showing only minor negative responses to the treatment. Subsequent exposure to simulated late spring frost increased the amount of damaged tissue, particularly in *S. polaris*, compared to the pre-frost situation. Wilting shoot tips of *S. polaris* increased nearly tenfold, while the proportion of wilted leaves of *L. confusa* increased by 15%. During recovery, damaged plants of *S. polaris* responded by extensive compensatory growth of new leaves that were much smaller than leaves of non-damaged shoots. The results suggest that *S. polaris* and *L. confusa* are rather tolerant to arctic winter-spring climate change, and this may be part of the reason for their wide distribution range and abundance in the Arctic.

Introduction

The climate of high-arctic regions is changing rapidly, particularly in wintertime (van Pelt *et al.* 2016, Vikhamar-Schuler *et al.* 2016, Overland *et*

al. 2017). The high-arctic archipelago of Svalbard has recently had extremely warm midwinters; for example, in January and February 2014 temperature was 11.2 to 14.5 °C above normal (Kristiansen *et al.* 2014). Such extreme changes

in winter temperature modify the snow conditions on the ground. Ice layers on the ground develop at Svalbard at increasing frequency, because winter climate is warming, resulting in more precipitation falling as rain, which freezes during subsequent cold periods (van Pelt *et al.* 2016). Several recent major icing events have taken place both at Svalbard and in other arctic regions (e.g. Hansen *et al.* 2014, Forbes *et al.* 2016, Bjerke *et al.* 2017a). Ground-ice is very detrimental to arctic animals, from small invertebrates (Coulson *et al.* 2000), via rodents (Callaghan *et al.* 2013) to large ungulates (Forchhammer and Boertmann 1993, Hansen *et al.* 2014).

However, the impacts of ground-ice on arctic vegetation are far from completely understood. While ice encasement of plants may lead to detrimental hypoxic conditions (Andrews 1996), the associated reduction of the snow cover and the low insulating properties of ice expose the plants to near-ambient fluctuations in temperature, which may also be detrimental (Kullman 1989, Bjerke *et al.* 2015). It has been proposed that high-arctic species are the most tolerant plants to ice encasement due to their lower rates of accumulation of anoxic metabolites (Crawford 1992, Crawford *et al.* 1994). However, evidence from experiments and natural events suggest that the arctic, alpine and boreal plants are sensitive to being encased in ice: the alpine snowbed forbs *Omalotheca supina* and *Sibbaldia procumbens* and the arctic chamaephyte *Cassiope tetragona* have low tolerance to ice encasement (Gudleifsson 2009, Milner *et al.* 2016), sub-arctic ecotypes of the chamaephytes *Empetrum nigrum* and *Vaccinium vitis-idaea* show reduced reproduction and increased electrolyte leakage under certain icing conditions (Preece and Phoenix 2014); while a natural icing event in a common garden caused extensive die-back of alpine plants from six continents (Bjerke *et al.* 2017b). Arctic-alpine lichens are intolerant of ice encapsulation at mild subfreezing temperature (Bjerke 2011) but show no mortality after being encapsulated in ice at temperature below -10°C (Bjerke 2009).

These few studies on the impacts of ground icing on arctic, alpine and northern boreal plants render important information, but more knowledge on icing impacts is required to fully understand the potential impacts of ongoing arctic

winter climate change (Bokhorst *et al.* 2016). In addition to midwinter impacts, warmer cold seasons also affect the winter–spring transition, causing advanced snowmelt, which may lead to earlier onset of the growing season. Early initiation of growing seasons is often associated with temperature backlashes and exposure to frost after deacclimation, and frost injuries to leaves and flowers have been recorded following early onset of growing seasons in alpine (e.g. Inouye 2008, Wheeler *et al.* 2014, 2016) and forest ecosystems (e.g. Jönsson and Bärning 2011, Hufkens *et al.* 2012).

Our experimental study seeks to determine the combined effects of ice encasement and frost exposure after onset of leafing on the performance of two widespread arctic plants, one prostrate shrub and one graminoid. The objective of this study was to determine the tolerance of the two high-arctic plant species to long-term (60 d) ice encasement. During the course of the experiment, we decided also to include a second stress treatment, late spring frost, to a subsample of the replicates that had been encased in ice. Damage ratios and physiological performance of surviving plants were assessed after a period of recovery. Thus, this study represents a hitherto untested combination of two winter-spring stress factors that are expected to increase in frequency. Dynamic global process-based vegetation modelling suggest that winter climate change will produce novel vegetation types at Svalbard and in other arctic regions (Reu *et al.* 2013). Thus, the results of this experiment will provide further insight to how arctic plants will cope with their rapid changes of their environment. Such information is required to fully understand the future vegetation composition of the Arctic.

Material and methods

Plant material

Salix polaris (polar willow) is a prostrate deciduous tundra shrub generally not taller than 3 cm. It forms a network of shoots on top of the soil, therefore often appearing as mat-forming. It is one of the most abundant vascular plant species at Svalbard (Rønning 1996) and has a near-

circumpolar distribution range and scattered occurrences in alpine regions south of the Arctic (Hultén and Fries 1986). It has a wide ecological amplitude ranging from mesic moss tundra to dry, gravelly, wind-exposed ridges. *Luzula confusa* (northern wood-rush) is a frequently occurring, short-statured rush growing on relatively dry, wind-exposed tundra at Svalbard and elsewhere in the Arctic (Rønning 1996, Elven 2016).

In August 2014, we collected seeds of these two species from dry, wind-exposed tundra at Svalbard, around seven monitoring sites between Bjørndalen and Bolterdalen in the vicinity of Longyearbyen (78.22°N, 15.71°E); cf. Bjerke *et al.* (2017a) for a map. The seeds were placed in paper bags, dried at 20 °C and transported to the climate laboratory Holt, Tromsø, in the sub-Arctic region of Norway (69.65°N, 18.91°E), where they were stored in freezers for 8 months. Seeds were mixed and potted. The potting soil was a 1:1 mixture of quartz-rich moraine sand retrieved from a gravel pit in Tønsvika in Tromsø and dried *Sphagnum* peat (“Veksttorv”, Tjerbo Torvfabrikk AS, Rakkestad, Norway). The peat had a pH between 5.5 and 6.5 and a nitrogen content of 850 mg l⁻¹, according to the manufacturer’s information. The nutrient content of the sand has not been analyzed in detail, but generally such leached quartz-rich glacial sand provides very little of any plant nutrient (Solomon and McShane 2015). Overall, the composition of the potting soil was selected to reflect the nutrient level of the soil in the plants’ natural habitat. The seeds were grown at 9 °C and low light (PAR < 200 μmol m⁻² s⁻¹). Seedlings were grown in a greenhouse at 9 °C with natural light from 6 June to 15 September. At the end of this period, *Luzula* plants were ca. 10 cm tall with a single plant per pot, while *Salix* plants, with 2–3 individuals per pot, had produced a network of shoots with 50 leaves or more per pot. It was no longer possible to distinguish individuals from each other. The pot was therefore the replicate unit for both species and is henceforth called “plant”. The germinability was lower than expected, resulting in fewer replicates than originally planned. Pots were moved outdoors for acclimation under natural autumn and early winter temperature and light conditions. Daily mean air temperature at Holt during this period

varied from 12.2 to –9.9 °C. The first snow covered the plants in mid-November, and by mid-December, the snow had accumulated to a depth of 38 cm, with a corresponding near-surface temperature (5 cm soil depth) at –0.1 °C. Snow cover was above 15 cm until early April, and temperature remained stable for the rest of the snow season, varying between 0.2 and –1.5 °C.

Simulation of ice encasement (IE)

In late January 2016, 29 pots of *S. polaris* and 54 pots of *L. confusa* with a 20 cm thick layer of natural snow were transferred to a dark chamber at 1 °C. Pot soil temperature was naturally frozen from outdoor conditions, being –0.5 °C at the time of transfer. The lower number of *S. polaris* pots was due to fewer germinating seeds of this species. Pots were placed in containers slightly larger and taller than the pots. Cold tap water was cooled to 0.5 °C and poured into the containers until plant parts were covered in water. Pots were immediately transferred to temperature-regulated freezers. Freezer temperature was set to –20 °C to allow for rapid ice development. When the water was almost completely frozen, temperature was increased to –2.5 °C at a rate of 5 °C h⁻¹. Plants were then kept at –2.5 °C for 60 d. When ice accumulates during arctic winters, it often lasts for 60 d or more (Coulson *et al.* 2000, Hansen *et al.* 2014). Therefore, this duration of ice encasement was considered as a realistic scenario. During this period, the plants were swapped between two freezers every 15 d to minimize freezer-specific effects on the treatment. During swapping, temperature in the freezers increased to ca. 3 °C for a short while, but this did not reduce the ice layer around the pots. Ten pots per species of *S. polaris* and *L. confusa* were not transferred in late January, but were kept outdoors under snow, as this is considered the optimal overwintering conditions for northern snow-adapted plants (Bokhorst *et al.* 2018), and these samples were therefore considered as controls.

The ice-encased pots were transferred to a dark chamber at 1 °C for thawing out. Control plants from under the snow were also moved to this temperature. After 3 d, the ice had melted from the ice-encased plants, while control plants

were free of snow. Plants were kept at 1 °C for another 3 d before the pots were transferred to a greenhouse with natural spring light and a temperature at 6–9 °C, varying with incoming solar radiation (maximum PAR around noon on clear days ca. 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Plants were grown under these conditions for 24 d. During this period, plant survival and leaf development were monitored.

Simulation of late spring frost (LSF)

Due to the low number of replicates, IE was the only planned treatment. However, observed high survival rate during the simulation of spring growth after IE, allowed us to add LSF to the experimental protocol by exposing 24 of the pots of *L. confusa* and 16 of the pots of *S. polaris* that had been encased in ice to this additional treatment. In late April 2016, these pots were returned to the freezer for simulation of late spring frost. Freezer temperature was lowered from 6 °C to –2.5 °C at a rate of 2 °C h⁻¹. Pots were kept at this temperature for 4 d before being returned to the greenhouse alongside the other pots. Since this was an unplanned treatment, it was not possible to include an LSF only treatment, i.e. LSF without a preceding IE treatment.

Measurements of plant vitality

All plants, including controls, were moved outdoors to the sub-Arctic laboratory garden and were grown for another 60 d (recovery period). During this period, plants were protected from direct sun for most of the day, this to avoid unnaturally high temperature exposure. The shade effect contributes to produce temperature regimes close to their natural high-Arctic habitat where solar radiation easily leads to surface temperature up to 10 °C above official weather station temperature which is measured at 2 m above ground (authors' own observations). Graae *et al.* (2012) show similar steep temperature gradients from alpine sites. Pots were watered regularly during the recovery period.

Plant performance was assessed after this recovery period. First, we assessed whether

plants were alive. Plants without any green cover were considered dead. For *S. polaris*, we measured the number and length of wilted top shoots, i.e. leafless top shoots on plants with leaves at basal stems. We also counted the number of healthy shoots, i.e. shoots without any visible signs of wilting. Control plants of both species were also assessed for signs of damage. All control plants were healthy without any signs of leaf wilting, but unexpectedly, had started autumn senescence earlier than the treated plants. Therefore, we did not measure leaf biomass of *L. confusa* controls, because leaves of these plants had reduced weight following autumn senescence. Moreover, we did not measure total green biomass, average leaf weight, chlorophyll concentration and flavonoid content of control plants of *S. polaris*, because some leaves had already been dropped, while the remaining leaves were autumn-coloured.

From the treatments IE and LSF, all leaf material of the two species was collected. For *S. polaris*, we counted the number of leaves per plant, and two randomly selected leaves per plant were analyzed immediately after collection using an optical plant performance instrument that measures the chlorophyll and flavonoid contents of the leaves (Dualox Scientific, Force A, Orsay, France). Instrument readings of chlorophyll were converted to concentration using the equation for dicots in Cerovic *et al.* (2012). Flavonoid content is presented in instrument units. Leaves of *L. confusa* were too narrow to be measured with this instrument. For *L. confusa*, we separated green leaf segments from wilted leaf segments. We placed the plant material of both species in paper bags and dried the material at 70 °C for 24 h and weighed the green and wilted leaves to the nearest 0.0001 g. For *S. polaris*, an average leaf weight per plant was calculated by dividing the total leaf weight with the number of leaves.

Statistics

All tests were run with SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). Data sets, i.e. all response variables described above, had strongly non-normal distributions. Therefore, the non-parametric Kruskal-Wallis test was applied

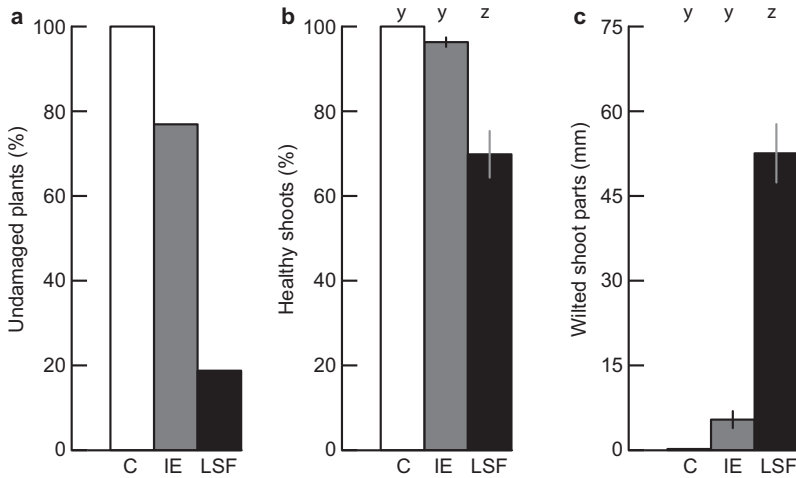


Fig. 1. Performance of *Salix polaris* after treatments. (a) Proportion of undamaged plants, (b) proportion of shoots without wilted tips, and (c) length of wilted shoot parts in control (C), ice encasement (IE) and late spring frost (LSF) treatments. Bars in b and c represent averages of 10 (C), 13 (IE) and 16 (LSF) replicates ± 1 standard error of the mean. Different letters above the bars indicate significant differences between treatments based on pairwise comparisons. Kruskal-Wallis test: **b:** $H = 17.81$, $p < 0.001$; **c:** $H = 20.47$, $p < 0.001$.

to test whether all treatment groups were equal at the 95% significance level. This test was applied in cases where we had three treatment groups, i.e. C, IE and LSF, as it is suitable for comparing three or more nonnormally distributed independent groups. We decided not to use Dunn's post-hoc test with sequential Bonferroni correction for pairwise comparisons, because this correction is overly conservative, needlessly increasing Type II error rates (Cabin and Mitchell 2000). There are also numerous mathematical, logical and practical objections against this method (Moran 2003). Thus, for pairwise comparisons, i.e. C vs. IE, C vs. LSF, and IE vs. LSF, we applied the Mann-Whitney *U*-test, which is suitable for comparing two independent, non-normally distributed samples. In the figures, significant differences between samples are highlighted using letters placed above bars. Treatments with different letters differ significantly ($p < 0.05$) from each other as determined by the Mann-Whitney *U* pairwise comparisons. Following the advice by Moran (2003), exact *p* values are provided in the text, allowing for individual judgement of their biological significance. The standardized test statistic *z* is provided for all Mann-Whitney *U*-test results. The number of replicates per species and treatment were as follows: *S. polaris*: C: $n = 10$, IE:

$n = 13$, LSF: $n = 16$; *L. confusa*: C: $n = 10$, IE: $n = 30$, LSF: $n = 24$.

Results

Ice encasement

All plants of *S. polaris* or *L. confusa* were alive after IE. 77% of the *S. polaris* plants did not show any signs of shoot damage following IE, while all the control plants were completely healthy, i.e. without any shoot damage (Fig. 1a). The ratio of healthy shoots and the length of wilted shoot parts in *S. polaris* following IE did not differ from control plants (Fig. 1b–c; Mann-Whitney *U*-test: healthy shoots: $z = 1.59$, $p = 0.38$; wilted shoot parts: $z = 1.59$, $p = 0.38$). The proportion of alive leaf biomass of *L. confusa* was reduced by 17 percentage points in IE as compared to control plants (Fig. 2a; $z = 4.58$, $p < 0.001$).

Late spring frost

The second stress treatment, which was LSF after leaf-out, led to increasing damage rates in

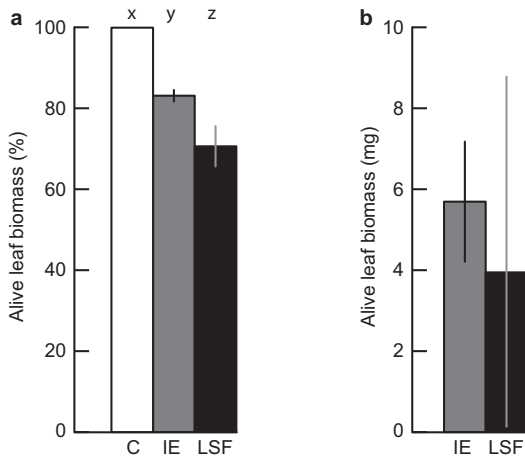


Fig. 2. Performance of *Luzula confusa* after treatments. a) Proportion of alive (green) leaf biomass after recovery period, b) amount of alive leaf biomass after recovery period in control (C), ice encasement (IE) and late spring frost (LSF) treatments. Amount of alive leaf biomass not measured in C; see text for more information). Bars represent averages of 10 (C), 30 (IE) and 24 (LSF) replicates ± 1 standard error of the mean. Different letters above the bars in 2a indicate significant differences between treatments based on pairwise comparisons. Kruskal-Wallis test: a: $H = 27.32$, $p < 0.001$.

both species. All plants of *S. polaris* survived LSF. However, the proportion of healthy plants was reduced by 58 percentage points from IE to LSF (Fig. 1a), while the proportion of healthy shoots decreased by 26 percentage points from IE to LSF (Fig. 1b; Mann-Whitney U -test: $z = 2.90$, $p = 0.005$). The number of total shoots did not differ between IE and LSF ($z = -0.79$, $p = 0.449$; data not shown). Total length of wilted shoot tips per plant was nearly tenfold higher after LSF than after IE (Fig. 1c; $z = 3.40$, $p = 0.001$). Total leaf biomass was reduced by 49% (Fig. 3a; $z = -2.24$, $p = 0.025$). Number of leaves varied greatly between plants of *S. polaris* and was not significantly different between IE and LSF ($z = -0.88$, $p = 0.398$; data not shown). This was partly due to extensive compensatory production of new leaves in damaged plants during the recovery phase. These new leaves were very small. Hence, mean leaf weight was reduced by 46% from IE to LSF (Fig. 3b; $z = -3.31$, $p < 0.001$). Chlorophyll content of *S. polaris* leaves did not differ between the two treatments (Fig. 3c; $z = -0.42$, $p = 0.682$), but flavonoid

content was 29% lower in LSF than in IE (Fig. 3d; $z = -3.53$, $p < 0.001$).

Eight percent of the *L. confusa* plants from the LSF treatment did not produce new leaves during the recovery phase and were defined as dead. After the recovery period, the proportion of alive leaf biomass was 12 percentage points lower in LSF than in IE, i.e. a 15% reduction (Fig. 2a; Mann-Whitney U -test: $z = 2.39$, $p = 0.017$). The amount of green biomass varied considerably between plants but was on average 31% lower after LSF than after IE (Fig. 2b; $z = -2.49$, $p = 0.013$).

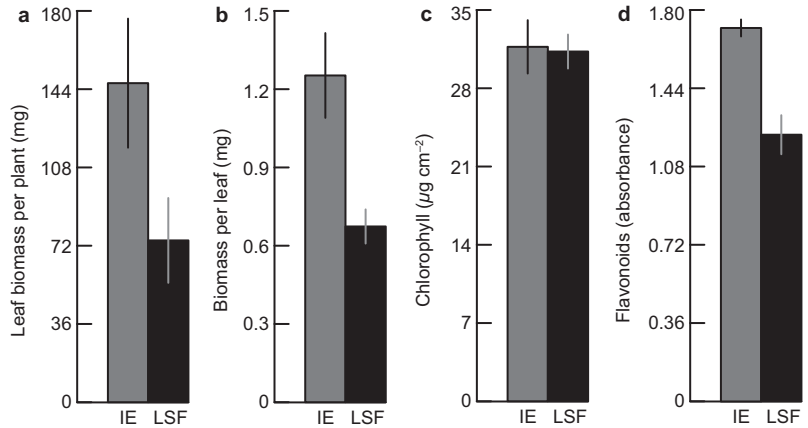
Discussion

The rather high tolerance of the high-arctic plants *Salix polaris* and *Luzula confusa* to anoxic ice encasement stands in contrast to previous ice encasement studies of arctic-alpine plants (Gudleifsson 2009, Milner *et al.* 2016). Our results are more in line with the results from summertime studies of high-arctic plants exposed to oxygen deprivation at 20 °C, which showed that leaves of Spitsbergen ecotypes of several species, including *L. confusa*, were highly anoxia-tolerant (Crawford *et al.* 1994). In combination, the study by Crawford *et al.* (1994) and the one presented here clearly show that *L. confusa* is tolerant to anoxic conditions, irrespective of the cause of anoxia.

Crawford *et al.* (1994) also tested the anoxia tolerance of *S. polaris*, elucidating that leaves were not tolerant, but buds were, and new leaves grew from buds during the recovery period. Our study indeed shows similar results. New leaves were produced after ice encasement, and the few plants with wilted top shoots, produced new leaves from the part of the stems that were still alive. The similarity in results between the two studies suggests that the oxygen deprivation method applied by Crawford *et al.* (1994) may be well suited also for testing arctic plants' tolerance to ice encasement.

The much lower survival rate reported for lichens encased in ice (Bjerke 2009, 2011) was assumed to be related to physiological activity; the lichens are physiologically active at mild subfreezing temperature, but this activity largely stops at temperature below -5 °C (Bjerke 2011).

Fig. 3. Comparison of *Salix polaris* (a) leaf biomass per plant (= pot), (b) biomass per leaf, (c) chlorophyll content, and (d) flavonoid content between ice encasement (IE) and late spring frost (LSF) treatments. Bars represent averages of 13 (IE) and 16 (LSF) replicates ± 1 standard error of the mean.



The differences in damage to vascular plants encased in ice may also be temperature-related. For example, *O. supina* and *S. procumbens* were treated at $-2\text{ }^{\circ}\text{C}$ (Gudleifsson 2009), which is warm enough to induce physiological activity, while *E. nigrum* and *V. vitis-idaea* were treated in the field under naturally variable temperature, which mostly remained below $-5\text{ }^{\circ}\text{C}$ and not varying much from the subnivean temperature in control plots because experimentally produced ice became covered in snow following snowy weather (Preece and Phoenix 2014).

Temperature during the treatment of *C. tetragona* was not shown in Milner *et al.* (2016), only temperature during the establishment, which was between -7 and $-15\text{ }^{\circ}\text{C}$. The iced plots were topped up with snow (Milner *et al.* 2016), something which would have reduced the effects of fluctuations in ambient temperature, probably leaving the encased plants at a stable temperature well below freezing. However, anoxia may have occurred in reactivated *C. tetragona* plants during spring thaw and caused the reported damage. In our study, we applied a mild subfreezing temperature ($-2.5\text{ }^{\circ}\text{C}$), because such temperature may impose more stress than colder temperature regimes, which result in full hibernation (Gudleifsson 2009, Bjerke 2011). Despite a very long period encased in ice followed by a long period of potential anoxia during thaw at $1\text{ }^{\circ}\text{C}$, the two species were in good health following IE; the treatment did not cause mortality at replicate level, only leading to a low ratio of wilting shoot tips.

The projected increase in extreme climatic events (Hov *et al.* 2013, Benestad *et al.* 2016) will expose ecosystems to a higher frequency of potentially stressful events per year and season (Smith 2011, Bjerke *et al.* 2014, Phoenix and Bjerke 2016). Thus, exposure to two potentially stressful events, one following shortly after the other, is a realistic scenario for these high-arctic plants, and this simulation shows that *L. confusa* and *S. polaris* were mildly to moderately damaged, with low (*L. confusa*) or no (*S. polaris*) mortality on replicate level. Both species responded by rapid compensatory growth. This suggests that a single winter-spring period with the combination of these two events will not affect much the abundance of these two species. However, compensatory growth under natural high-arctic conditions may be slower than that experienced in this experiment, despite our efforts to simulate natural conditions, both regarding soil nutrient levels and ground temperature, during the recovery phase. Previous experiments suggest that associated functional groups, including competing lichens and dwarf shrubs, would suffer from higher dieback ratios (Gudleifsson 2009, Bjerke 2011, Milner *et al.* 2016). Evidence from recent extreme winter perturbation events at Svalbard, which exposed plants to fluctuating ambient winter temperature and/or encased plants in ice, suggest that *C. tetragona* is one of the plants that is most sensitive to a changing winter climate, while *L. confusa* and *S. polaris* were not among the species displaying visible damage in the field (Bjerke *et*

al. 2017a). Hence, *L. confusa* and *S. polaris* may be able to increase their relative abundance at the expense of associated species with which they compete for space. However, even if damage is not easily visible to the naked eye, these species may respond non-visually, for example through reduced growth rates, as shown from tree ring measurements of *S. polaris* following a series of stressful winters with rain falling on snow (Opała-Owczarek *et al.* 2018).

The measurements of chlorophyll and epidermal flavonoid content of *S. polaris* show some interesting contrasts between IE and LSF. While flavonoid content differed between the two treatments, chlorophyll content did not. The chlorophyll measurements show that leaves were potentially equally productive after IE and LSF, despite the higher damage ratio at shoot level following LSF. Thus, LSF plants were able to transport water and nutrients to developing leaves, suggesting that there was no physiological damage to the shoot parts that did not wilt. Epidermal flavonoids are synthesized as a response to increased light (Cerovic *et al.* 2012), in northern regions often in combination with cool temperatures (Oberbauer and Starr 2002). Flavonoid concentrations often decline in autumn (Bjerke *et al.* 2018). A reduced flavonoid content may therefore suggest that plants exposed to LSF initiated autumn senescence earlier than the IE plants.

The high survival and rapid compensatory growth of *L. confusa* and *S. polaris* after IE may be keys to their wide distribution and dominance. However, a series of cold seasons with such events recurring yearly may also affect growth (Opała-Owczarek *et al.* 2018) and the longer-term abundance of *L. confusa* and *S. polaris*. If such a dramatic situation will take place, it is currently unclear which functional types would be able to increase their abundance. To elucidate this, more species, including various types of bryophytes and lichens, would be necessary to test experimentally.

It was unfortunate that there was not enough plant material to include a separate LSF treatment to test the contrasts between IE + LSF and LSF only. We would assume that an LSF only treatment would have resulted in fewer negative effects than IE + LSF. It was also unfortunate that control plants were not studied in similar

detail as treated plants. However, assessment of plant and shoot survival of control plants clearly suggest that that IE led to modest effects, while IE + LSF caused stronger effects.

Overall, our study shows that the two studied species are rather tolerant to stress during the cold season, but that they differ slightly in their tolerance to both ice encasement and the subsequent exposure to late spring frost. *Salix polaris* was more tolerant than *L. confusa*, showing no dieback at plant level, only at shoot level, while *L. confusa* had an 8% plant dieback after LSF. Comparison with other sources of information suggests that these two species are among the most tolerant species to winter climate change in the Arctic.

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References

- Andrews C.J. 1996. How do plants survive ice? *Annals of Botany* 78: 529–536.
- Benestad R.E., Parding K.M., Isaksen K. & Mezghani A. 2016. Climate change and projections for the Barents region: what is expected to change and what will stay the same? *Environmental Research Letters* 11, 054017, doi:10.1088/1748-9326/11/5/054017.
- Bjerke J.W. 2009. Ice encapsulation rather protects than disturbs the freezing lichen. *Plant Biology* 11: 227–235.
- Bjerke J.W. 2011. Winter climate change: Ice encapsulation at mild subfreezing temperatures kills freeze-tolerant lichens. *Environmental and Experimental Botany* 72: 404–408.
- Bjerke J.W., Karlsen S.R., Høgda K.A., Malnes E., Jepsen J.U., Lovibond S., Vikhamar-Schuler D. & Tømmervik H. 2014. Record-low primary productivity and high plant damage in the Nordic Arctic Region in 2012 caused by multiple weather events and pest outbreak. *Environmental Research Letters* 9, 084006, doi:10.1088/1748-9326/10/9/095007.
- Bjerke J.W., Tømmervik H., Zielke M. & Jørgensen M. 2015. Impacts of snow season on ground-ice accumulation, soil frost and primary productivity in a grassland of sub-arctic Norway. *Environmental Research Letters* 10, 095007, doi:10.1088/1748-9326/10/9/095007.

- Bjerke J.W., Treharne R., Vikhamar-Schuler D., Karlsen S.R., Ravolainen V., Bokhorst S., Phoenix G.K., Bochenek Z. & Tømmervik H. 2017a. Understanding the drivers of extensive plant damage in boreal and Arctic ecosystems: Insights from field surveys in the aftermath of damage. *Science of the Total Environment* 599–600: 1965–1976.
- Bjerke J.W., Elvebakk A. & Tømmervik H. 2017b. Alpine garden plants from six continents show high vulnerability to ice encasement. *Norwegian Journal of Geography* 72: 57–64.
- Bjerke J.W., Wierzbinski G., Tømmervik H., Phoenix G.K. & Bokhorst S. 2018. Stress-induced secondary leaves of a boreal deciduous shrub (*Vaccinium myrtillus*) overwinter then regain activity the following growing season. *Nordic Journal of Botany* 36: e01894, doi:10.1111/njb.01894.
- Bokhorst S., Pedersen S.H., Brucker L., Anisimov O., Bjerke J.W., Brown R.D., Ehrlich D., Essery R.L.H., Heilig A., Ingvander S., Johansson C., Johansson M., Jónsdóttir I.S., Niila I., Luojus K., Macelloni G., Mariash H., McLennan D., Rosqvist G.N., Sato A., Savela H., Schneebeli M., Sokolov A., Sokratov S.A., Terzago S., Vikhamar-Schuler D., Williamson S.N., Qiu Y. & Callaghan T.V. 2016. Changing arctic snow cover: A review of recent developments and assessment of future needs for observations, modelling and impacts. *Ambio* 45: 516–537.
- Bokhorst S., Jaakola L., Karppinen K., Edvinsen G.K., Mæhre H.K. & Bjerke J.W. 2018. Contrasting survival and physiological responses of sub-Arctic plant types to extreme winter warming and nitrogen. *Planta* 247: 635–648.
- Callaghan T.V., Jonasson C., Thierfelder T., Zhenlin Y., Hedenäs H., Johansson M., Molau U., van Bogaert R., Michelsen A., Olofsson J., Gwynn-Jones D., Bokhorst S., Phoenix G.K., Bjerke J.W., Tømmervik H., Christensen T.R., Hanna E., Koller E.K. & Sloan V.L. 2013. Ecosystem change and stability over multiple decades in the Swedish sub-arctic: complex processes and multiple drivers. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368, 20120488, doi:10.1098/rstb.2012.0488.
- Cabin R.J. & Mitchell R.J. 2000. To Bonferroni or not to Bonferroni: when and how are the questions. *Bulletin of the Ecological Society of America* 81: 246–248.
- Cerovic Z.G., Masdoumier G., Ghozlen B.N. & Latouche G. 2012. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiologia Plantarum* 146: 251–260.
- Coulson S.J., Leinaas H.P., Ims R.A. & Søvik G. 2000. Experimental manipulation of the winter surface ice layer: the effects on a high arctic soil microarthropod community. *Ecography* 23: 299–306.
- Crawford R.M.M. 1992. Oxygen availability as an ecological limit to plant distribution. *Advances in Ecological Research* 23: 93–185.
- Crawford R.M.M., Chapman H.M. & Hodge H. 1994. Anoxia tolerance in high arctic vegetation. *Arctic and Alpine Research* 26: 308–312.
- Elven R. (ed.) 2016. *Annotated checklist of the panarctic flora (PAF): vascular plants*. Available at <http://panarcticflora.org/>.
- Forbes B.C., Kumpula T., Meschytyb N., Laptander R., Macias-Fauria M., Zetterberg P., Verdonen M., Skarin A., Kim K.Y., Boisvert L.N., Stroeve J.C. & Bartsch A. 2016. Sea ice, rain-on-snow and tundra reindeer nomadism in arctic Russia. *Biology Letters* 12, 20160466, doi:10.1098/rsbl.2016.0466.
- Forchhammer M. & Boertmann D. 1993. The muskoxen *Ovibos moschatus* in north and northeast Greenland – population trends and the influence of abiotic parameters on population dynamics. *Ecography* 16: 299–308.
- Graae B.J., De Frenne P., Kolb A., Brunet J., Chabrerie O., Verheyen K., Pepin N., Heinken T., Zobel M., Shevtsova A., Nijs I. & Milbau A. 2012. On the use of weather data in ecological studies along altitudinal and latitudinal gradients. *Oikos* 121: 3–19.
- Gudleifsson B.E. 2009. Ice encasement damage on grass crops and alpine plants in Iceland — impact of climate change. In: Gusta L., Wisniewski M. & Tanino K. (eds.), *Plant cold hardiness: from the laboratory to the field*, CABI, Wallingford, pp. 163–172.
- Hansen B.B., Isaksen K., Benestad R.E., Kohler J., Pedersen Å.Ø., Loe L.E., Coulson S.J., Larsen J.O. & Varpe Ø. 2014. Warmer and wetter winters: characteristics and implications of an extreme weather event in the high Arctic. *Environmental Research Letters* 9, 114021, doi:10.1088/1748-9326/9/11/114021.
- Hov Ø., Cubasch U., Fischer E., Höpfe P., Iversen T., Kvamstø N.G., Kundzewicz Z.W., Rezacova D., Rios D., Santos F.D., Schädler B., Veisz O., Zerefos C., Benestad R., Murlis J., Donat M., Leckebusch G.C. & Ulbrich U. 2013. *Extreme weather events in Europe: preparing for climate change adaptation*. Norwegian Meteorological Institute, Oslo.
- Hufkens K., Friedl M.A., Keenan T.F., Sonnentag O., Bailey A., O’Keefe J. & Richardson A.D. 2012. Ecological impacts of a widespread frost event following early spring leaf-out. *Global Change Biology* 18: 2365–2377.
- Hultén E. & Fries M. 1986. *Atlas of North European vascular plants: north of the Tropic of Cancer I-III*. Koeltz Scientific Books, Königstein.
- Inouye D. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89: 353–362.
- Jönsson A.M. & Barring L. 2011. Ensemble analysis of frost damage on vegetation caused by spring backlashes in a warmer Europe. *Natural Hazards and Earth System Sciences* 11: 401–418.
- Kristiansen S., Mamen J., Szewcyk-Bartnicka H. & Tajet H.T.T. 2014. *Været i Norge. Klimatologisk oversikt, januar–februar 2014*. Available at <https://www.met.no/publikasjoner/met-info/met-info-2014>.
- Kullman L. 1989. Cold-induced dieback of montane spruce forests in the Swedish Scandes — a modern analogue of paleoenvironmental processes. *New Phytologist* 113: 377–389.
- Milner J.M., Varpe Ø., van der Wal R. & Hansen B.B. 2016. Experimental icing affects growth, mortality, and flowering in a high arctic dwarf shrub. *Ecology and Evolution*

- 6: 2139–2148.
- Moran M.D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* 100: 403–405.
- Oberbauer S.F. & Starr G. 2002. The role of anthocyanins for photosynthesis of Alaskan Arctic evergreens during snowmelt. *Advances in Botanical Research* 37: 129–145.
- Opala-Owczarek M., Piroznikow E., Owczarek P., Szymański W., Luks B., Kępski D., Szymanowski M., Wojtuń B. & Migala K. 2018. The influence of abiotic factors on the growth of two vascular plant species (*Saxifraga oppositifolia* and *Salix Polar*) in the High Arctic. *Catena* 163: 219–232.
- Overland J., Walsh J. & Kattsov V. 2017. Trends and feedbacks. In: *Snow, water, ice and permafrost in the Arctic (SWIPA) 2017*, Arctic Monitoring and Assessment Programme, Oslo, pp. 9–23.
- Phoenix G.K. & Bjerke J.W. 2016. Arctic browning: events and trends as drivers. *Global Change Biology* 22: 2960–2962.
- Preece C. & Phoenix G.K. 2014. Impact of early and late winter icing events on sub-arctic dwarf shrubs. *Plant Biology* 16: 125–132.
- Reu B., Zaehle S., Bohn K., Pavlick R., Schmidtlein S., Williams J.W. & Kleidon A. 2014. Future no-analogue vegetation produced by no-analogue combinations of temperature and insolation. *Global Ecology and Biogeography* 23: 156–167.
- Rønning O.I. 1996. *The flora of Svalbard*, 3rd ed. Norwegian Polar Institute, Oslo.
- Smith M.D. 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *Journal of Ecology* 90: 656–663.
- Solomon S. & McShane M. 2015. *Growing vegetables west of the Cascades*. Sasquatch Books, Seattle.
- van Pelt W.J.J., Kohler J., Liston G.E., Hagen J.O.M., Luks B., Reijmer C.H. & Pohjola V.A. 2016. Multi-decadal climate and seasonal snow conditions in Svalbard. *Journal of Geophysical Research, Earth Surface* 121: 2100–2117.
- Vikhamar-Schuler D., Isaksen K., Haugen J.E., Tømmervik H., Luks B., Schuler T.V. & Bjerke J.W. 2016. Changes in winter warming events in the Nordic Arctic Region. *Journal of Climate* 29: 6223–6244.
- Wheeler J.A., Hoch G., Cortés A.J., Sedlacek J., Wipf S. & Rixen C. 2014. Increased spring freezing vulnerability for alpine shrubs under early snowmelt. *Oecologia* 175: 219–229.
- Wheeler J.A., Cortés A.J., Sedlacek J., Karrenberg S., van Kleunen M., Wipf S., Hoch G., Bossdorf O. & Rixen C. 2016. The snow and the willows: earlier spring snowmelt reduces performance in the low-lying alpine shrub *Salix herbacea*. *Journal of Ecology* 104: 1041–1050.