Seedling responses to decreased snow depend on canopy composition and small-mammal herbivore presence

Running head: Seedling responses to snow and herbivory

Peter W. Guiden[1]*
Brian M. Connolly[1,2]
John L. Orrock[1]

[1]Department of Integrative Biology, University of Wisconsin-Madison, 250 N. Mills St, Madison WI 53706

[2]Current address: Department of Biology, Eastern Michigan University, 441 Mark Jefferson Science Complex, Ypsilanti MI 48198

*Corresponding author. Phone: (937) 545-8926, E-mail: guiden@wisc.edu

Abstract

Winter is becoming warmer and shorter across the northern hemisphere, but reductions in snow can decrease tree seedling survival by exposing seedlings to harmful winter microclimates. Similarly, herbivory by small mammals can also limit the survival and distribution of woody plants, but it is unclear whether winter climate change will alter small-mammal herbivory. Although small-scale experiments show that snow removal can either increase or decrease both soil temperatures and herbivory, we currently lack large-scale snow-removal experiments needed to predict the effect of reduced snow. To understand how winter herbivory and snow conditions influence seedling dynamics, we transplanted *Acer saccharum* and *Tsuga canadensis* seedlings across a 180 km latitudinal gradient in northern Wisconsin, where snow depth varied seven-fold among sites. Seedlings were transplanted into one of two herbivory treatments (small-mammal exclosure, small-mammal access) and one of two late-winter snow removal treatments (snow removed, snow unmanipulated). Snow removal increased soil freeze-thaw frequency and cumulative growing degree-days (GDD), but the magnitude of these effects depended on forest canopy composition. *Acer saccharum* survival decreased where snow was removed, but only at sites without conifers. Excluding small mammals increased *A. saccharum* survival at sites where the small-mammal herbivore *Myodes gapperi* was present. Excluding small mammals also increased *T. canadensis* survival in plots with < 5 cm snow. Because variation in canopy composition and *M. gapperi* presence were important predictors of seedling survival across the snow-depth gradient, these results may provide a way to make more accurate predictions of winter seedling survival over large spatial scales. Global change scenarios that project future patterns of seedling recruitment may benefit from explicitly considering interactions between snow conditions and small-mammal winter herbivory.

Key words: northern temperate forest; *Myodes gapperi*; winter climate change

Introduction

Climate change is projected to alter patterns of forest biodiversity by shifting the ranges of many species poleward (Parmesan et al. 1999, Iverson et al. 2008), but observational evidence suggests the ranges of many temperate tree species are contracting or not shifting (Zhu et al. 2012). Discrepancies between model projections and observational data may arise because abiotic conditions (e.g., freezing events) and biotic interactions (e.g., herbivory) can limit tree seedling survival and performance, either alone or in concert (Tylianakis et al. 2008, Brown and Vellend 2014, Urban et al. 2016). For example, hot and dry conditions may directly increase tree mortality (Chmura et al. 2011), but may also increase herbivory, resulting in landscape-wide changes in plant survival (Raffa et al. 2008). Despite the possibility that climate and herbivory can interact to generate non-intuitive shifts in consumer pressure (Brown and Vellend 2014), few studies examine changes in herbivory along climatic gradients, such as snow depth, which represents a key knowledge gap in efforts to predict changes in community dynamics under future climate scenarios.

Climate and climate-mediated changes in herbivory may both have particularly strong effects in temperate ecosystems, where snow cover directly influences overwinter plant survival (Tessier 2017) and also moderates herbivory (Swihart and Bryant 2001, Roy et al. 2004, Martin and Maron 2012, Christenson et al. 2014). These effects are important to understand because warming winter temperatures are decreasing snow depth and snow cover across the northern hemisphere (Dye 2002, Henry 2008, Brown and Robinson 2011), possibly exposing plants to damaging freeze-thaw events that lead to mortality or delayed leaf-out (Tierney et al. 2001, Inouye 2008, Kreyling et al. 2010). Predicting the effect of winter climate change on plant-herbivore interactions is difficult because snow cover can have multiple effects on herbivores, and the relative magnitude of these effects is poorly understood. Small mammals, such as the southern red-backed vole (*Myodes gapperi*), can be important consumers of seeds or seedlings in temperate ecosystems during winter (Haken and Batzli 1996, Ostfeld et al. 1997, Howe and Brown 2000, Manson et al. 2001, Korslund and Steen 2006, Sullivan and Sullivan 2008), and snow cover may facilitate herbivory by small mammals by providing a refuge from predation or harsh temperatures (Kausrud et al. 2008, Pauli et al. 2013, Sultaire et al. 2016, Sanders-DeMott et al. *in press*). However, small mammals may alternatively find seeds and seedlings more readily in areas with less snow (i.e., reduced snow increases food apparency; Anderson 1986, Shimano and Masuzawa 1998). Because of the potential for interactions among regional-scale abiotic gradients in winter conditions and the strength of small-mammal herbivory, experiments that manipulate snow depth and small-mammal access should be replicated across large spatial scales in order to understand the unique and interactive contributions of winter climate and herbivores to large-scale patterns in tree seedling survival and performance (Maron et al. 2014, Penczykowski et al. 2017).

In this study, we examined the response of seedlings to experimental manipulations of snow depth and small-mammal herbivory along a natural gradient in snow accumulation (Notaro et al. 2011). Using exclosures that manipulated small-mammal access, we monitored the effects of small-mammal herbivory and experimental snow removal on seedling survival, phenology, and growth of two regionally important tree species (*Acer saccharum* and *Tsuga canadensis*). We hypothesized that snow removal would increase late-winter soil temperature variability, reducing seedling survival and delaying leaf-out of deciduous seedlings. We also expected snow depth to influence winter herbivory by small mammals, leading us to test two alternative hypotheses: a) snow removal would reduce small-mammal herbivory by eliminating important winter habitat and restricting herbivore movement (e.g., Korslund and Steen 2006), and b) snow removal would increase small-mammal herbivory by increasing seedling apparency (e.g., Anderson 1986).

Materials and Methods

Study area and focal species

This work was conducted at ten sites spanning a 180 km latitudinal gradient in northern Wisconsin, USA (44.55 to 46.14 °N, Figure 1A). Long-term winter temperature and precipitation summaries for this region are provided in Appendix S1. Sites along this gradient vary in canopy composition (Curtis 1959) and small-mammal community composition (Stephens and Anderson 2014), but variation in canopy and small-mammal community composition are independent of variation in snow depth (Appendix S2, Appendix S3). Each 18 x 12 m site was subdivided into four 12 x 1.5 m plots. Common tree species in these forests included (in descending order of basal area) *Acer saccharum* (Sapindaceae), *Quercus rubra* (Fagaceae), *Abies balsamea* (Pinaceae), *Acer rubrum* (Sapindaceae), and *Tsuga canadensis* (Pinaceae). Winter soil temperatures can differ considerably between coniferous and deciduous forests (Petty et al. 2015), so we recorded the presence or absence of conifers at each site. Conifers were present at four sites. Because the abundance of small mammals can strongly influence tree seedling survival (Gill 1992), we quantified variation in the small mammal community of each site using live-trapping (Appendix S2). *Myodes gapperi* was present at half of the sites (n = 5).

We investigated the response of seedlings of two tree species, *A. saccharum* and *T. canadensis*, to winter herbivory and decreased snow depth. These species are projected to decline in importance throughout northern temperate forests in the next century as mean temperatures warm (Iverson et al. 2008), and anecdotal evidence suggests that these seedlings may be vulnerable to winter herbivory by voles (Cleavitt et al. 2014). *Acer saccharum* and *T. canadensis* seeds were obtained from the Wisconsin Department of Natural Resources Griffith State Nursery (Wisconsin Rapids, WI). All seeds were cold-stratified at 1°C for eight weeks, after which they were placed in a growth chamber (model E-41L2, Percival Scientific, Perry IA) programmed to a 12-hour 25°/15°C, light/dark cycle to induce germination (Burns and Honkala 1990). Emerging seedlings were transferred to containers with potting medium (Metromix 366, Sun Gro Horticulture, Agawam, MA) in a greenhouse, where they received supplemental light and daily watering. Seedlings also received fertilizer (Peters Professional 20-10-20, Everris NA, Inc. Dublin, OH) 3-4 times per week at 380 ppm nitrogen. Because we continuously transferred seedlings to the greenhouse, seedling age at the time of transplant ranged from approximately four to six weeks, but seedling age was stratified by treatment and site.

Experimental design

Within each field plot, we installed two open-top cylindrical small-mammal exclosures in June 2015, for a total of 8 exclosures at each site (Figure 1B; for details about exclosure design, see Appendix S4). On 20-21 July 2015, we transplanted 80 *A. saccharum* and 58 *T. canadensis* seedlings into the exclosures (maximum of 1 seedling per species per exclosure), and did not give seedlings any supplemental water or fertilizer. The lower number of *T. canadensis* seedlings was a result of low germination success. Immediately before transplanting, we measured the aboveground stem height of each seedling. We placed a small wooden popsicle stick approximately 5 cm away from seedlings to help relocate seedlings (Schnurr et al. 2004). We recorded seedling survival in September 2015, November 2015, June 2016, and September 2016. Seedlings were considered alive if stem and leaf tissue were both present. Seedlings were also observed in March, April, and May 2016, but because we could not distinguish dead seedlings from those that had not yet leafed out, we did not model seedling survival at those times, although these data were used to quantify the effect of snow removal and winter herbivory on seedling phenology.

On November 6-7, 2015, we conducted an herbivory experiment to investigate the importance of small mammal herbivory during winter in northern temperate forests (Figure 1B). One of two exclosures per plot was designated as a control treatment, in which seedlings experienced no herbivory. A second exclosure was used to manipulate small-mammal herbivore access, which had two 7 x 7 cm holes to allow small mammals to freely enter the exclosures and consume seedlings. By placing seedlings in an "open" exclosure, we created a pseudo-exclosure treatment that controlled for potential differences in microclimate elicited by our exclosure design (Radtke and Wilson 2015).

Starting in February 2016, we experimentally removed snow to evaluate the role of snow cover in affecting seedling survival and small-mammal herbivory. Adjacent plots were paired (n = 3 plot pairs per site), and within each pair one plot was randomly designated to have snow removed (Figure 1B). We removed snow by shoveling until snow was 5 cm deep (Hardy et al. 2001, Drescher and Thomas 2013, Christenson et al. 2014); see Appendix S5 for additional information. The second plot was designated as a control with ambient (i.e., unmanipulated) snow depth. This resulted in a split-split-plot design, where herbivore treatment was nested within snow removal treatment, which was nested within site. Although many snow-removal treatments remove snow throughout the entire winter (e.g., Groffman et al. 2001, Drescher and Thomas 2013), we restricted our shoveling treatment to three sessions in the late winter and early spring of 2016 to mimic the most likely winter climate change scenarios in this region (Brown and Robinson 2011, Notaro et al. 2011): mid-February (February 19-21), early March (March 4-5), and mid-March (March 18). In order to track differences in snow accumulation among sites, two snow-depth measurements were made in each plot with a graded polyvinyl chloride (PVC) pole every two weeks between December 2015 through March 2016. While we reduced snow depth in order to manipulate the availability of subnivium habitat, changing soil temperatures and herbivore behavior (Pauli et al. 2013, Penczykowski et al. 2017), it is important to note that snow removal also potentially reduces soil water and nutrient availability (Hardy et al. 2001).

Statistical analysis

We quantified the effect of snow removal on snow accumulation and soil temperatures across the ten sites in this study area using linear regression. For each snow removal session (mid-February, early March, mid-March), we modeled the mean snow depth in each plot after removal as a function of snow removal treatment and shoveling session, using a linear mixed model with a random intercept term for site. To understand how our snow-removal treatment altered soil temperature, we modelled the response of cumulative growing-degree days (GDD), using a growing threshold of 10°C (Raulier and Bernier 2000), and the frequency of freeze-thaw events to snow removal using MANOVA. Subsequently, we modeled the response of soil temperature variables to snow removal with univariate ANOVA. Because conifers can have a strong influence on winter soil temperatures (Petty et al. 2015), we included the presence or absence of conifers in the canopy as a covariate in univariate models (Appendix S3).

We used Kaplan-Meier survival analysis (“survival” package in R; Therneau and Grambsch 2000) to evaluate the time course of survival across the four periods when seedlings were checked in the field. This provided a seasonal estimate of survival, but we were unable to detect differences in the time course of survival between herbivory and snow-removal treatments due to the relatively low number of sampling points. In order to understand the interactive effects of reduced snow cover and small-mammal herbivory on seedling survival, we constructed a generalized linear mixed model with a binomial error structure ("lme4" package in R; Bates et al. 2015). We used seedling survival at the end of the study (September 2016) as the response variable, but using June 2016 seedling survival did not change our conclusions. We tested the effect of small-mammal winter herbivory by modeling *Acer saccharum* seedling survival as a function of snow removal treatment (ambient snow, snow removed), herbivory treatment (small-mammal access, small-mammal exclosure), *M. gapperi* presence or absence, and all possible interactions. Our model also accounted for seedling responses to changes in microclimate caused by snow removal by including the presence or absence of conifers at each site (which had a strong effect on soil temperatures, see Results and Appendix S2), as well as a snow removal × conifer interaction. Finally, we included snow depth after shoveling (measured in early March, to capture peak differences in snow depth among sites), and a snow depth × herbivory treatment interaction in order to investigate how the strength of small-mammal herbivory changed across the snow depth gradient encompassed at our sites. *Tsuga canadensis* survival was modeled similarly, but due to the low number of surviving *T. canadensis* seedlings (see Results), models with three-way interactions did not converge, so we report only main effects and significant two-way interactions.

In addition to *Acer saccharum* seedling survival, we modeled the response of leaf phenology to winter herbivory and snow conditions in surviving seedlings. We did not model *Tsuga canadensis* phenology due to low numbers of surviving individuals. We recorded seedling leaf phenology in March 2016, April 2016, mid-May 2016, and late May 2016 by classifying each *A. saccharum* seedling into one of three categories based on the most advanced stage present: dormant, buds opening, or new leaf tissue visible (Wesołowski and Rowiński 2006). For analysis, we used the most conservative approach possible and only considered seedlings with new, unfolded leaf tissue visible as having initiated leaf-out. Seedling leaf-out was modeled as a binomial variable at each of the four sampling dates. This model was structured similarly to the model of survival described above, but included sampling date as an ordinal predictor variable and a random intercept for each unique seedling.

We also measured stem height of all species (measured as the distance between the apical meristem and the ground) in June 2016, as *A. saccharum* stem growth is typically completed for the season at this time (Burns and Honkala 1990). To understand how snow removal and herbivory affected seedling growth, we calculated relative stem growth rate for each seedling. Relative stem growth rate, calculated as the difference of log-transformed stem height in 2016 from log-transformed stem height in 2015, divided by the time interval between measurements (t = 0.96 years), accounts for differences in growth rate due to differences in initial size or study duration (Gibson 2002). Seedling growth was modeled as a linear mixed-effects model in the "lme4" package in R, using similar model structures as described for models of survival. In all survival and performance models, a random intercept term was included for plot nested within site to account for our split-plot design (Schielzeth and Nakagawa 2013). All analyses were conducted in R (R Core Team 2017), and post-hoc pairwise comparisons were conducted using the "lsmeans" package in R (Lenth 2016). Detailed results for all mixed-effects models are provided in Appendix S6.

Results

The winter of 2015-2016 was abnormally warm and had below-average snowfall (Appendix S1). Across the latitudinal gradient encompassed by our sites, we observed the greatest variation in ambient snow depth in early March (range: 6.3 to 42.2 cm; Figure 2A). In addition to this natural snow depth gradient, our shoveling treatment reduced snow depth by an average of 15.7 ± 3.4 cm in snow-removal plots in mid-February and 15.2 ± 3.8 cm in early March (Figure 2A). Differences in snow depth between snow-removal and ambient-snow plots largely disappeared by mid-March with the onset of spring, when shoveling only reduced snow depth by 1.1 ± 0.5 cm (Figure 2A). During the 42 days between the initiation of our snow-removal treatment and final snow retreat, our snow removal treatment increased both freeze-thaw frequency and growing degree-days (MANOVA: $F_{2,35} = 17.59$, $P < 0.0001$). Univariate tests showed that snow-removal plots experienced 72% more freeze-thaw events (12.93 ± 1.94 freeze-thaw events) compared to plots with ambient snow depth (7.53 ± 1.08 freeze-thaw events, $F_{1,24.6} = 22.30$, $P < 0.001$), but snow removal increased freeze-thaw frequency the most at sites with conifers (snow removal × conifer interaction: $F_{1,24.6} = 7.69$, $P = 0.01$, Appendix S3). Additionally, snow-removal plots also accumulated 92% more growing degree-days (5.09 ± 0.43) compared to plots with ambient snow depth (2.66 ± 0.22, $F_{1,26.7} = 69.94$, $P < 0.001$), which was driven by an increase in GDD at sites with no conifers (snow removal × conifer interaction: $F_{1,26.6} = 59.92$, $P < 0.001$, Appendix S3). Whereas unmanipulated control plots were characterized by either high GDD or high freeze-thaw frequency depending on the presence of conifers, snow-removal plots experienced a combination of both high GDD and high freeze-thaw frequency that was rarely seen in plots with ambient snow depth (Figure 2B).

Patterns of seedling survival differed considerably between *A. saccharum* and *T. canadensis*. At the conclusion of the experiment (September 2016), *A. saccharum* seedling survival was relatively high (0.561 ± 0.045, Kaplan-Meier estimator ± standard error), whereas *T. canadensis* seedling survival was almost an order of magnitude lower (0.067 ± 0.026). The time interval encompassing winter and spring represented the greatest period of seedling mortality for both species. *Acer saccharum* survival decreased from 0.952 ± 0.023 in November 2015 to 0.771 ± 0.046 in June 2016 (n = 15 deaths, $\chi^2 = 4.18$, d.f. = 1, $P = 0.04$) and *T. canadensis* survival decreased from 0.617 ± 0.063 in November 2015 to 0.200 ± 0.052 in June 2016 (n = 25 deaths, $\chi^2 =136.5$, d.f. = 1, $P < 0.001$).

Winter microclimate and small-mammal herbivory were important predictors of *A. saccharum* seedling survival, as demonstrated by a significant interaction between *M. gapperi* presence and herbivory treatment ($\chi^2 = 5.18$, d.f. = 1, $P = 0.02$, Figure 3A). This interaction arose because seedling survival in the herbivore-access treatment was lower at sites where *M. gapperi* were captured compared to sites where *M. gapperi* were not captured (pairwise comparison: z = 2.64, $P = 0.04$). Seedling survival in herbivore exclosures did not differ between sites with and without *M. gapperi* (pairwise comparison: z = 1.41, $P = 0.50$). There was no main effect of small-mammal exclosures ($\chi^2 = 0.12$, d.f. = 1, $P = 0.73$) or *M. gapperi* presence ($\chi^2 = 1.37$, d.f. = 1, $P = 0.24$) on seedling survival. Seedling survival was reduced in plots with snow removed, but only at sites without conifers (snow removal × conifer: $\chi^2 = 3.87$, d.f. = 1, $P = 0.05$, Figure 4A). There was no significant main effect of snow removal ($\chi^2 = 1.55$, d.f. = 1, $P = 0.21$) or conifers ($\chi^2 = 0.18$, d.f. = 1, $P = 0.67$). *Acer saccharum* survival was not affected by snow depth after shoveling ($\chi^2 = 0.06$, d.f. = 1, $P = 0.81$), or a snow depth × herbivory treatment interaction ($\chi^2 = 0.71$, d.f. = 1, $P = 0.40$).

While *T. canadensis* survival was low throughout the study area, winter herbivory and snow conditions may explain some variation in *T. canadensis* seedling survival. *Tsuga canadensis* survival increased in plots with low snow depth, but only where small-mammal herbivores were excluded ($\chi^2 = 5.23$, d.f. = 1, $P = 0.02$, Figure 3B). There was a marginally significant main effect of herbivory treatment (χ^2 = 1.64, d.f. = 1, *P* = 0.06), suggesting that survival was approximately three times greater in small-mammal exclosures (0.178 ± 0.073) compared to the small-mammal access treatment (0.067 ± 0.046). There was no significant main effect of snow depth on *T. canadensis* seedling survival (χ^2 = 0.13, d.f. = 1, *P* = 0.72). Tsuga canadensis survival was not affected by snow removal (χ^2 = 0.01, d.f. = 1, *P* = 0.92), conifers (χ^2 = 2.68, d.f. = 1, *P* = 0.11), *M. gapperi* presence (χ^2 = 0.17, d.f. = 1, *P* = 0.68).

Acer saccharum seedlings were more likely to leaf out at later sampling dates (χ^2 = 58.00, d.f = 1, *P* < 0.0001), but snow conditions and herbivory had a strong effect on the timing of seedling leaf-out. A significant snow depth × herbivory interaction suggested that small-mammal herbivory delayed leaf-out, but only in plots with shallow snow (χ^2 = 4.56, d.f. = 1, *P* = 0.03, Figure 3C). Seedlings in small-mammal exclosures leafed out earlier on average than seedlings in the small-mammal access treatment (χ^2 = 5.18, d.f. = 1, *P* = 0.02), but there was no significant main effect of snow depth after shoveling on seedling leaf-out date (χ^2 = 0.65, d.f. = 1, *P* = 0.42). Delayed leaf-out in the herbivore access treatment was driven by seedlings with obvious signs of stem damage (Appendix S7). Additionally, a significant snow removal × conifer interaction (χ^2 = 3.65, d.f. = 1, *P* = 0.05) and main effect of conifers (χ^2 = 10.84,d.f. = 1, *P* = 0.0009) suggested that seedlings leafed out later in sites with conifers, particularly in plots with snow removed (Figure 4B). However, leaf-out date was unaffected by the main effect of snow removal (χ^2 = 0.75, d.f. = 1, *P* = 0.39), snow depth after shoveling (χ^2 = 0.65, d.f. = 1, *P* = 0.42), or *M. gapperi* presence (χ^2 = 0.75, d.f. = 1, *P* = 0.39), and there was no significant snow removal × herbivory interaction (χ^2 = 0.80, d.f. = 1, *P* = 0.37), snow removal × *M. gapperi* presence interaction (χ^2 = 0.15, d.f. = 1, *P* = 0.70), herbivory × conifer interaction (χ^2 = 0.07, d.f. = 1, *P* = 0.79) or herbivory × *M. gapperi* presence interaction ($\chi^2 = 1.11$, $P = 0.29$). We found no effect of snow removal or herbivore exclosure on seedling growth (Appendix S6).

Discussion

Projected shifts in winter climate may alter snow cover and snow depth in northern temperate ecosystems (Notaro et al. 2011), potentially changing the survival and distribution of tree seedlings by modifying plant-herbivore interactions. Using an experiment that manipulated snow cover and winter herbivory across sites that varied substantially in snowfall, we found evidence that winter herbivory by small mammals reduced *A. saccharum* and *T. canadensis* survival and delayed *A. saccharum* leaf-out, and that these negative effects were often strongest in areas characterized by shallow snow (Figure 3). We also found that snow removal decreased seedling survival at sites with conifers, but delayed leaf-out at sites with conifers (Figure 4). Three implications of these results are discussed below. First, reduced snow depth might change foraging behavior of small-mammal herbivores in temperate forests by increasing seedling apparency. Second, by altering seedling phenology, winter herbivory and winter climate change might indirectly shape subsequent plant-herbivore interactions in spring and summer. Third, predicting the ecological effects of reduced snow cover will require an understanding of factors influencing local soil temperatures. These findings suggest that winter herbivory and snow conditions can have important effects on seedling dynamics observed in summer, but that the direction and magnitude of the effects of winter climate change can vary substantially across landscapes.

Snow depth drives variation in small-mammal winter herbivory

In boreal systems, deep snow generally increases small-mammal herbivory by decreasing predation risk or providing a stable subnivium habitat where small mammals can locate seedlings

(Korslund and Steen 2006, Kausrud et al. 2008, Penczykowski et al. 2017). However, in the temperate forests we studied, we found evidence that negative effects of small-mammal herbivory were strongest in shallow snow. Herbivore exclosures in shallow snow marginally increased *T. canadensis* survival (Figure 3B) and prevented *A. saccharum* seedling damage that was associated with delayed leaf-out (Figure 3C, Appendix S7). We suggest that these patterns were observed because in a historically warm winter, where subnivium formation was rare, snow removal primarily altered the apparency of seedlings to small mammal herbivores (i.e., seedlings were more likely to be discovered by small-mammal herbivores where snow was shallow). Snow has been shown to reduce seedling apparency to large ungulate herbivores (e.g., Christenson et al. 2014), and anecdotal evidence from temperate systems suggest that snow cover may also obscure seeds from small mammals (Anderson 1986, Shimano and Masuzawa 1998). Greater within-site replication of seedlings in future studies could improve our understanding of the importance of increased seedling apparency in years of high versus low seedling density that characterize the mast cycle of many temperate tree species (Manson et al. 1998).

While large ungulate herbivores can limit *A. saccharum* and *T. canadensis* seedling survival (e.g., Salk et al. 2011), our work demonstrates that voles can also be important seedling herbivores in temperate forests. *Myodes gapperi* presence was an important predictor of *A. saccharum* seedling mortality in herbivore access plots (Figure 3A, Appendix S7), suggesting that land managers could expect decreased natural seedling recruitment in habitats with *M. gapperi*, particularly in years with shallow snow cover. The weak effect of *M. gapperi* presence on *T. canadensis* survival suggests that low *T. canadensis* seedling survival, likely driven by unfavorable climatic conditions, reduced the number of *T. canadensis* seedlings available for *M. gapperi* to encounter. However, even after escaping herbivory as seedlings, trees may face *M.*

gapperi herbivory as saplings that could delay phenology or decrease survival (Sanders-DeMott, et al. in press). While we found important negative effects of *M. gapperi* herbivory, warming temperatures in this region have been associated with declines in *M. gapperi* relative abundance (Myers et al. 2009). Therefore, it will be important to consider how winter climate change will alter seedling herbivory by both voles and other herbivore guilds that typically benefit from reduced snow, such as large ungulates (Murray et al. 2014).

Winter climate and herbivory may affect plant-herbivore dynamics in the growing season

Understanding links between winter conditions and summer seedling performance could improve our ability to predict conditions that cause herbivores to limit plant establishment (Patankar et al. 2013), helping to resolve the considerable variation often observed in plant-herbivore interactions (Agrawal 2011). While reduced snow and winter herbivory can be important causes of plant mortality (Howe and Brown 2000, Schaberg et al. 2008, Martin and Maron 2012, Drescher and Thomas 2013, Drescher 2014), the potential for variation in winter climates to influence herbivory of surviving individuals is less understood (Connolly et al. 2017). Here, we show that winter herbivory and variable winter soil temperatures may delay *A. saccharum* seedling leaf-out, and suggest that seedlings that survive harsh winter conditions could alter herbivory in the growing season.

We found evidence that leaf-out in deciduous tree seedlings can be delayed by both winter herbivory (Figure 3B, Appendix S7; Herder et al. 2009), and variable winter temperatures (Figure 4B; Bokhorst et al. 2011). While damage from either winter herbivory or winter climate may have strong lethal effects on seedlings (Figure 3A, Figure 4A, Appendix S7), damaged seedlings that survive winter may suffer subtler sublethal effects. For example, leaf phenology can play a key role in plant-herbivore interactions in spring and summer by either increasing or decreasing the temporal overlap of invertebrate herbivores and expanding seedling leaves (Boege and Marquis 2005). Understanding phenological shifts by both seedlings (Figure 4B) and invertebrate herbivores (Pureswaran et al. 2014) to changing winter temperatures could provide important insights about how winter climate change modifies herbivory in the growing season. Additionally, winter injuries can alter the morphology and chemistry of leaves produced in spring. Injured seedlings may produce poorly defended leaves that are more susceptible to invertebrate herbivory (Herder et al. 2009, Patankar et al. 2013, Rubert-Nason et al. 2017), or low-quality leaves that are avoided by herbivores (St. Clair et al. 2009). Future studies that focus on the effect of winter injuries on plant phenology, and in particular how phenology may shape seedling defense (Bryant 2003), could identify patterns in what we currently interpret as idiosyncratic responses of spring and summer herbivory to variation in winter climate (Roy et al. 2004).

Spatial variation in the effects of snow removal

Climate velocity, the rate and direction of climate change, may vary across relatively short spatial scales (e.g., Ordonez and Williams 2013), making it difficult to predict the ecological consequences of climate change for plant communities. For example, warmer winters with reduced snow can either increase the duration of soil freezing and the frequency of damaging freeze-thaw events (e.g., Tierney et al. 2001), or increase the length of the growing season (e.g., Sherwood et al. 2017). The large spatial extent of our study helps resolve previously observed discrepancies in snow removal experiments by showing that the effects of snow removal can vary along canopy composition gradients (Appendix S2): removing snow at sites without conifers increased cumulative GDD, but removing snow at sites with conifers increased freeze-thaw frequency (Figure 2B). Consequently, reduced snow depth may have lethal effects on seedlings in some habitats, but sublethal effects in other habitats. While many temperate species can tolerate extremely low temperatures, seedlings may respond to warmer winter temperatures by decreasing investment in cold tolerance, and thus repeated freeze-thaw cycles can decrease seedling survival (Figure 4A, Kreyling 2010). In habitats with fewer freeze-thaw events, such as sites with conifers in our study area, milder injuries caused by reduced snow depth may be more likely to delay the initiation of growth, rather than kill seedlings outright (Figure 4B, Blume-Werry et al. 2016). However, because this experiment was conducted in a historically warm winter, these results may not inform efforts to predict the response of soil temperatures or seedling dynamics to reduced snow depth in winters that characterized by historically average temperatures. By combining canopy data with other proxies for winter temperatures, such as topography or proximity to lakes (Ordonez and Williams 2013), it may be possible for land managers to identify areas that are most likely to experience potentially dangerous winter soil temperatures in future climates characterized by reduced snow, and prioritize resources to protect seedlings in those microsites (Schaberg et al. 2008).

Adaptations to winter temperature variability may increase the survival and performance of southern tree species relative to northern tree species in sites where seedlings experience an early growing season together with increased freeze-thaw frequency. Our study area represents the northern end of the *A. saccharum* distribution, but marks the southern end of the *T. canadensis* distribution (Burns and Honkala 1990, Fisichelli et al. 2014). Frequent freeze-thaw events may contribute to recruitment failure in relict *T. canadensis* populations (Friesner and Potzger 1944), but freeze-thaw events are relatively common throughout most of the current *A. saccharum* distribution (Hershfield 1974, Burns and Honkala 1990). Species that are poorly adapted to freeze-thaw events, including *T. canadensis*, may benefit from active management (e.g., insulating soil near seedlings) where winter temperature or precipitation preclude the formation of a subnivium (Schaberg et al. 2008), particularly in low-mast years.

Conclusions

Snow depth can be a key predictor of northern temperate forest composition (Henne et al. 2007), and might also affect the strength of winter herbivory by changing herbivore behavior (Fisichelli et al. 2012, Martin and Maron 2012). Despite calls to integrate species interactions into models of future plant species distributions (e.g., Urban et al. 2016), the potential for reduced snow cover to limit seedling establishment is not often explicitly considered in efforts to map projected range shifts. Here, we show that the effects of winter climate change on soil temperatures, and consequently seedling dynamics, will likely vary across regional landscapes, and that snow depth may play an underappreciated role in shaping winter herbivory by small mammals. This work suggests several new directions for future research. While our work demonstrates an important interaction between snow cover and small-mammal herbivory, climate change will likely alter northern temperate forest snow conditions at larger spatial and temporal extents than we manipulated here. Snow removal experiments could be conducted at larger spatial scales that approach small-mammal home ranges in winter (15 m x 15 m, Merritt and Merritt 1978) for several years (Blume-Werry et al. 2016) to capture fluctuations in winter temperatures, seedling density, and small-mammal population size. Additionally, variation in canopy composition may provide a means of predicting the effects of reduced snow on winter soil temperatures, and consequently seedling survival, at large spatial scales. While ecologists grapple with the challenge of predicting species distributions and abundances in a rapidly changing world, understanding the intricate links between winter and summer processes could provide an important perspective in predicting the future composition of northern temperate forests.

Acknowledgements

This work was made possible by outstanding logistical support from Trout Lake Field Station. We thank J. Casper, L. Maillefer, and M. Rowe for field assistance. S. Bartel, B. Bron, J. Chandler, R. Eddy, and H. Howe provided valuable feedback on the manuscript. This work was funded by an AFRI-NIFA Fellowship (Grant # 2014-02074) awarded to JLO and BMC, as well as a Vilas Associates Fellowship awarded to JLO. PWG was supported by a National Science Foundation IGERT award (Grant # DGE-1144752) while writing this manuscript as well as Doctoral Dissertation Improvement Grant funding from NSF (Grant # DEB-1701506).

Author contributions: PWG, BMC, and JLO contributed to experimental design; PWG and BMC collected data; PWG performed all analyses and led preparation of the manuscript; BMC and JLO helped revise the manuscript.

Data accessibility: If this manuscript is accepted for publication, relevant data supporting the results will be archived in a publicly accessible database (e.g., Figshare).

Literature Cited

Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence. − Func. Ecol. 25:420–432.

Anderson, P. K. 1986. Foraging range in mice and voles: the role of risk. − Can. J. Zoo. 64:2645–2653.

Bates, D. et al. 2015. Fitting linear mixed-effects models usling lme4. – J. of Stat. Softw. 67:1–48.

Blume-Werry, G., et al. 2016. Short-term climate change manipulation effects do not scale up to long-term legacies: effects of an absent snow cover on boreal forest plants. − J. Ecol. 104:1638–1648.

Boege, K., and Marquis, R. J. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. − Trends Ecol. Evo. 20:441–448.

Bokhorst, S., et al. 2011. Impacts of multiple extreme winter warming events on sub-Arctic heathland: phenology, reproduction, growth, and CO_2 flux responses. − Global Change Biol. 17:2817–2830.

Brown, C. D., and M. Vellend. 2014. Non-climatic constraints on upper elevational plant range expansion under climate change. − Proc. Roy. Soc. B: Biol. Sci. 281:20141779.

Brown, R. D., and Robinson, D. A. 2011. Northern hemisphere spring snow cover variability and change over 1922-2010 including an assessment of uncertainty. − Cryosphere 5:219–229.

Bryant, J.P. 2003. Winter browsing on Alaska feltleaf willow twigs improves leaf nutritional value for snowshoe hares in summer. − Oikos 102:25–32.

Burns, R. M., and Honkala, B. H. 1990. Silvics of North America. Second edition. U.S. Department of Agriculture, Forest Service.

Chmura, D. J., et al. 2011. Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. – For. Ecol. Manage. 261:1121–1142.

Christenson, L. M., et al. 2014. Cascading effects of climate change on forest ecosystems: biogeochemical links between trees and moose in the northeast USA. − Ecosystems 17:442–457.

Cleavitt, N.L., et al. (2014). Determinants of survival over 7 years for a natural cohort of sugar maple seedlings in a northern hardwood forest. − J. For. Res. 44:1112–1121.

Connolly, B. M., et al. 2017. Past freeze – thaw events on *Pinus* seeds increase seedling herbivory. − Ecosphere 8:e01748.

Curtis, J. T. 1959. The vegetation of Wisconsin: an ordination of plant communities. Second edition. University of Wisconsin Press.

Drescher, M. 2014. Snow cover manipulations and passive warming affect post-winter seed germination: a case study of three cold-temperate tree species. – Clim. Res. 60:175–186.

Drescher, M., and Thomas, S. C. 2013. Snow cover manipulations alter survival of early life stages of cold-temperate tree species. − Oikos 122:541–554.

Dye, D. G. 2002. Variability and trends in the annual snow-cover cycle in Northern Hemisphere land areas, 1972-2000. – Hydrol. Proc. 16:3065–3077.

Fisichelli, N., et al. 2012. Sapling growth responses to warmer temperatures "cooled" by browse pressure. − Global Change Biol. 18:3455–3463.

Fisichelli, N., et al. 2014. First-year seedlings and climate change: species-specific responses of 15 North American tree species. − Oikos 123:1331–1340.

Friesner, R. C., and Potzger, J. E. 1944. Survival of hemlock seedlings in a relict colony under forest conditions. − Butler Univ. Bot. Studies 6:102–115.

Gibson, D. 2002. Methods in comparative plant population ecology. Oxford University Press.

Gill, R. M. A. 1992. A review of damage by mammals in North temperate forests. − Forestry 65:145–169.

Groffman, P. M., et al. 2001. Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. – Biogeochem. 56:191–213.

Haken, A. E., and Batzli, G. O. 1996. Effects of availability of food and interspecific competition on diets of prairie voles. − J. Mamm. 77:315–324.

Hardy, J. P., et al. 2001. Snow depth manipulation and its influence on soil frost and water dynamics in a northern hardwood forest. – Biogeochem. 56:151–174.

Henne, P. D., et al. 2007. Lake-effect snow as the dominant control of mesic-forest distribution in Michigan, USA. − J. Ecol. 95:517–529.

Henry, H.A.L. 2008. Climate change and soil freezing dynamics: historical trends and projected changes. − Clim. Change 87: 421–434.

Herder, M., et al. (2009). Effects of natural winter browsing and simulated summer browsing by moose on growth and shoot biomass of birch and its associated invertebrate fauna. − Ann. Zoo. Fen., 46:63–74.

Hershfield, D. M. 1974. The frequency of freeze-thaw cycles. − J. Appl. Met. 13:348–354.

Howe, H. F., and Brown, J. S. 2000. Early effects of rodent granivory on experimental forb communities. – Ecol. Appl. 10:917–924.

Inouye, D.W. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. − Ecology 89: 353–62.

Iverson, L. R., et al. 2008. Estimating potential habitat for 134 eastern US tree species under six climate scenarios. − For. Ecol. Manage. 254:390–406.

Kausrud, K. L., et al. 2008. Linking climate change to lemming cycles. − Nature 456:93–7.

Korslund, L., and Steen, H. 2006. Small rodent winter survival: snow conditions limit access to food resources. − J. Anim. Ecol. 75:156–166.

Kreyling 2010. Winter climate change: a critical factor for temperate vegetation performance. – Ecology. 91:1939-1948.

Kreyling, J., et al. 2010. Effects of soil freeze-thaw cycles differ between experimental plant communities. − Basic Appl. Ecol. 11:65–75.

Lenth, R. (2016). Least-squares means: the R package lsmeans. J. Stat. Softw., 69, 1–33.

Manson, R. H. et al. 1998. The effects of tree seed and seedling density on predation rates by rodents in old fields. − Ecoscience 5:183–190.

Manson, R. H., et al. 2001. Long-term effects of rodent herbivores on tree invasion dynamics along forest-field edges. − Ecology 82:3320–3329.

Maron, J. L., et al. 2014. Disentangling the drivers of context-dependent plant-animal interactions. – J. Ecol. 102:1485–1496.

Martin, T. E., and Maron, J. L. 2012. Climate impacts on bird and plant communities from altered animal–plant interactions. − Nat. Clim. Change 2:195–200.

Merritt, J. F. and Merritt, J.M. 1978. Seasonal home ranges and activity of small mammals of a Colorado subalpine forest. − Acta Theri. 23: 195–202.

Murray, B. D., et al. 2014. A migratory ungulate facilitates cross-boundary nitrogen transport in forested landscapes. − Ecosystems 17:1002–1013.

Myers, P., et al. 2009. Climate-induced changes in the small mammal communities of the northern Great Lakes region. − Global Change Biol. 15:1434–1454.

Notaro, M., et al. 2011. 21st century Wisconsin snow projections based on an operational snow model driven by statistically downscaled climate data. – Inter. J. Clim. 31:1615–1633.

Ordonez, A., and Williams, J. W. 2013. Projected climate reshuffling based on multivariate climate-availability, climate-analog, and climate-velocity analyses: implications for community disaggregation. − Clim. Change 119:659–675.

Ostfeld, R. S., et al. 1997. Effects of rodents on survival of tree seeds and seedlings invading old fields. − Ecology 78:1531–1542.

Parmesan, C., et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. − Nature 399:579–583.

Patankar, R., et al. Permafrost-driven differences in habitat quality determine plant response to gall-inducing mite herbivory. − J. Ecol. 101:1042–1052.

Pauli, J. N., et al. 2013. The subnivium: a deteriorating seasonal refugium. − Front. Ecol. Env. 11:260–267.

Penczykowski, et al. 2017. Winter is changing: Trophic interactions under altered snow regimes. − Food Webs 13:80-91.

Petty, S. K., et al. 2015. Winter conditions and land cover structure the subnivium, a seasonal refuge beneath the snow. − PLOS One 10:e0127613.

Radtke, T. M., and Wilson, S. D. 2015. A limited role for apparent competition via granivory in the persistence of a grassland invader. – J. Veg. Sci. 26:995–1004.

Raffa, K. F., et al. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. – BioSci. 58:501.

Raulier, F., and Bernier, P. Y. 2000. Predicting the date of leaf emergence for sugar maple across its native range. − Can. J. For. Res. 30:1429–1435.

Roy, B. A., et al. 2004. Response of plant pathogens and herbivores to a warming experiment. − Ecology 85:2570–2581.

Rubert-Nason, K.F., et al. 2017. Vernal freeze damage and genetic variation alter tree growth, chemistry, and insect interactions. − Plant Cell Env., 40, 2743–2753.

Salk, T. T., et al. 2011. Poor recruitment is changing the structure and species composition of an old-growth hemlock-hardwood forest. − For. Ecol. Manage. 261:1998–2006.

Sanders-DeMott, et al. (*in press*). Snow depth, soil temperature, and plant-herbivore interactions mediate plant response to climate change. − J. Ecol.

Schaberg, P. G., et al. 2008. Influence of simulated snow cover on the cold tolerance and freezing injury of yellow-cedar seedlings. − Global Change Biol. 14:1282–1293.

Schielzeth, H., and Nakagawa, S. 2013. Nested by design: model fitting and interpretation in a mixed model era. – Meth. Ecol. Evo. 4:14–24.

Schnurr, J., et al. 2004. Neighborhood analyses of small-mammal dynamics: impacts on seed predation and seedling establishment. − Ecology 85:741–755.

Sherwood, J.A., et al. 2017. Effects of experimentally reduced snowpack and passive warming on montane meadow plant phenology and floral resources. − Ecosphere 8, e01745.

Shimano, K., and Masuzawa, T. 1998. Effects of snow accumulation on survival of beech (*Fagus crenata*) seed. − Plant Ecol. 134:235–241.

St. Clair, S.B., et al. 2009. Altered leaf morphology, leaf resource dilution and defense chemistry induction in frost-defoliated aspen (*Populus tremuloides*). − Tree Phys. 29:1259–1268.

Stephens, R. B., and Anderson, E. M. 2014. Habitat associations and assemblages of small mammals in natural plant communities of Wisconsin. – J. Mamm. 95:404–420.

Sullivan, T. P., and Sullivan, D. S. 2008. Vole-feeding damage and forest plantation protection: large-scale application of diversionary food to reduce damage to newly planted trees. − Crop Prot. 27:775–784.

Sultaire, S. M., et al. 2016. Climate change surpasses land-use change in the contracting range boundary of a winter-adapted mammal. − Proc. Roy. Soc. B: Biol. Sci. 283:20153104.

Swihart, R. K., and Bryant, J. P. 2001. Importance of biogeography and ontogeny of woody plants in winter herbivory by mammals. – J. Mamm. 82:1–21.

Tessier, J. T. 2017. Importance of depth in soil to corm survival in *Erythronium americanum* (Liliaceae). − Rhodora 119:33–43.

Therneau, T. M., and Grambsch, P. M. 2000. Modeling survival data: extending the cox model. Springer.

Tierney, G. L., et al. 2001. Soil freezing alters fine root dynamics in a northern hardwood forest. − Biogeochem. 56:175–190.

Tylianakis, J. M., et al. 2008. Global change and species interactions in terrestrial ecosystems. – Ecol. Letters 11:1351–1363.

Urban, M. C., et al. 2016. Improving the forecast for biodiversity under climate change. − Science 353:aad8466.

Wesołowski, T., and Rowiński, P. 2006. Timing of bud burst and tree-leaf development in a multispecies temperate forest. – For. Ecol. Manage. 237:387–393.

Zhu, K., et al. Failure to migrate: lack of tree range expansion in response to climate change. − Global Change Biol. 18:1042–1052.

Figure legends

Figure 1: A) Map of study area. Triangles represent the 10 sites used in this study and are color-coded to show variation in ambient snow depth. B) Experimental design used at each site to manipulate snow depth and winter herbivory.

Figure 2: A) Changes in snow depth over time in snow-removal plots (gold) and unmanipulated control plots (purple). Snow removal began 17 February 2016 (dashed line). B) Biplot of cumulative soil growing degree-days (10°C threshold) and freeze-thaw frequency in snow-removal plots (gold triangles) and unmanipulated control plots (purple circles). Large points with error bars represent least-squares means ± one standard error for each snow removal treatment.

Figure 3: Winter herbivory by small mammals influenced seedling survival and phenology. A) *Acer saccharum* seedling survival decreased at sites with *Myodes gapperi* (southern red-backed vole), but only in plots that allowed herbivore access (green circles). Points represent least-square means ± one standard error. B) *Tsuga canadensis* seedling survival increased in plots with shallow snow, but only in small-mammal herbivore exclosures (orange triangles). Curves represent estimated response variable values for herbivore access (green lines) and herbivore exclosures (orange lines). Points represent raw data spread out to reduce point overlap.

C) *Acer saccharum* leaf-out occurred earlier in shallow snow, but only where herbivores were excluded. Points represent least-square means ± one standard error.

Figure 4: Winter microclimate had strong effects on *Acer saccharum* survival and phenology. A) Seedling survival decreased in plots with snow removed, but only at sites where conifers were absent. Points represent least-square means ± one standard error. B) Seedling leaf-out occurred later in plots with snow removed, but only at sites with conifers.

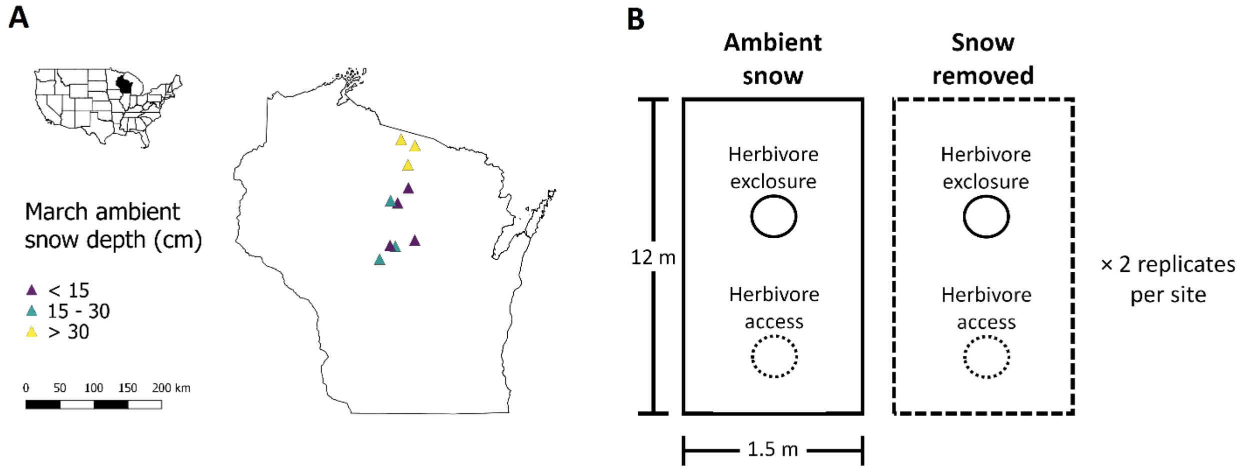

A
March ambient snow depth (cm)
▲ < 15
▲ 15 - 30
▲ > 30
0 50 100 150 200 km
B
Ambient snow
Snow removed
Herbivore exclosure
Herbivore access
Herbivore exclosure
Herbivore access
12 m
1.5 m
× 2 replicates per site

Figure 1

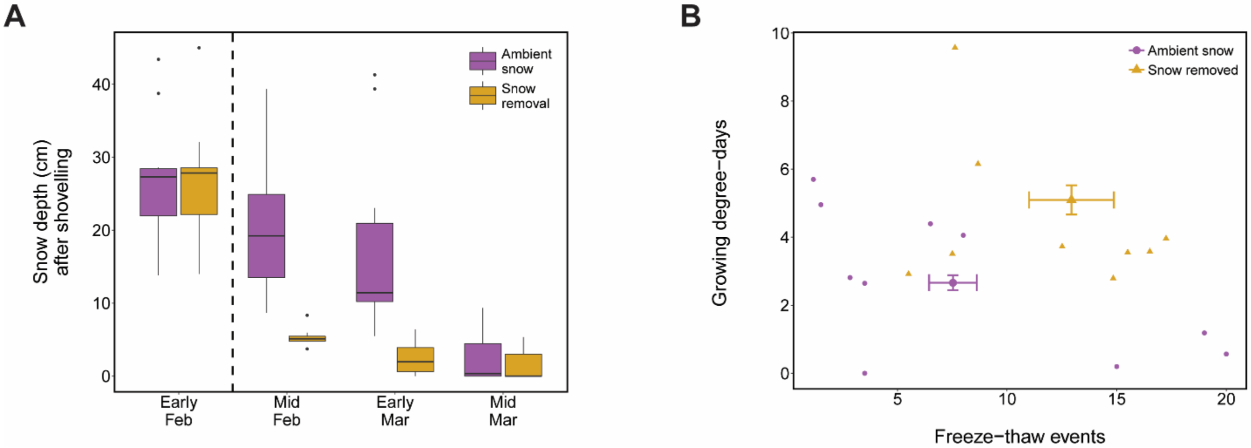
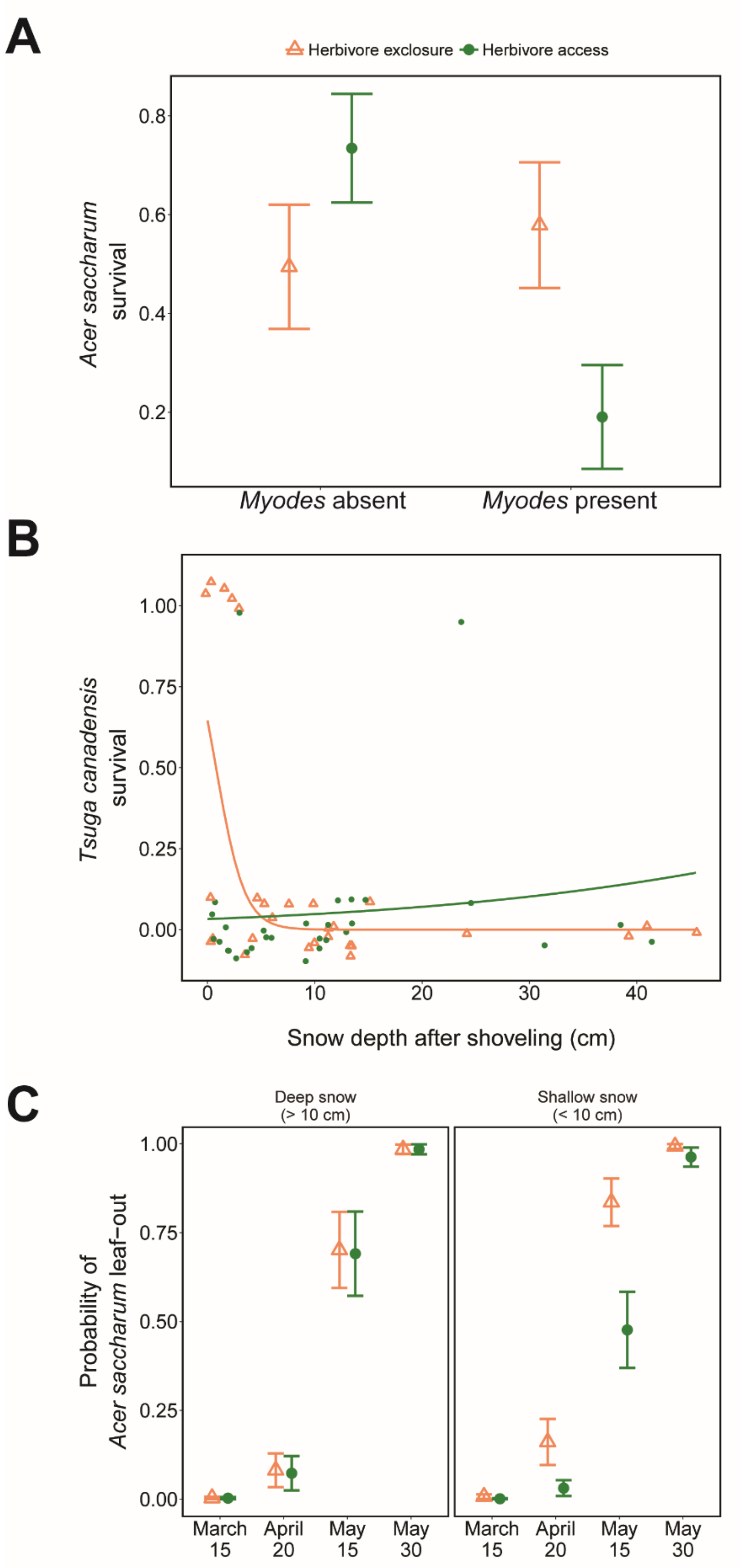

A
Snow depth (cm) after shovelling
0
10
20
30
40
Ambient snow
Snow removal
Early Feb
Mid Feb
Early Mar
Mid Mar
B
Growing degree−days
0
2
4
6
8
10
Ambient snow
Snow removed
5
10
15
20
Freeze−thaw events

Figure 2

634



635 Figure 3

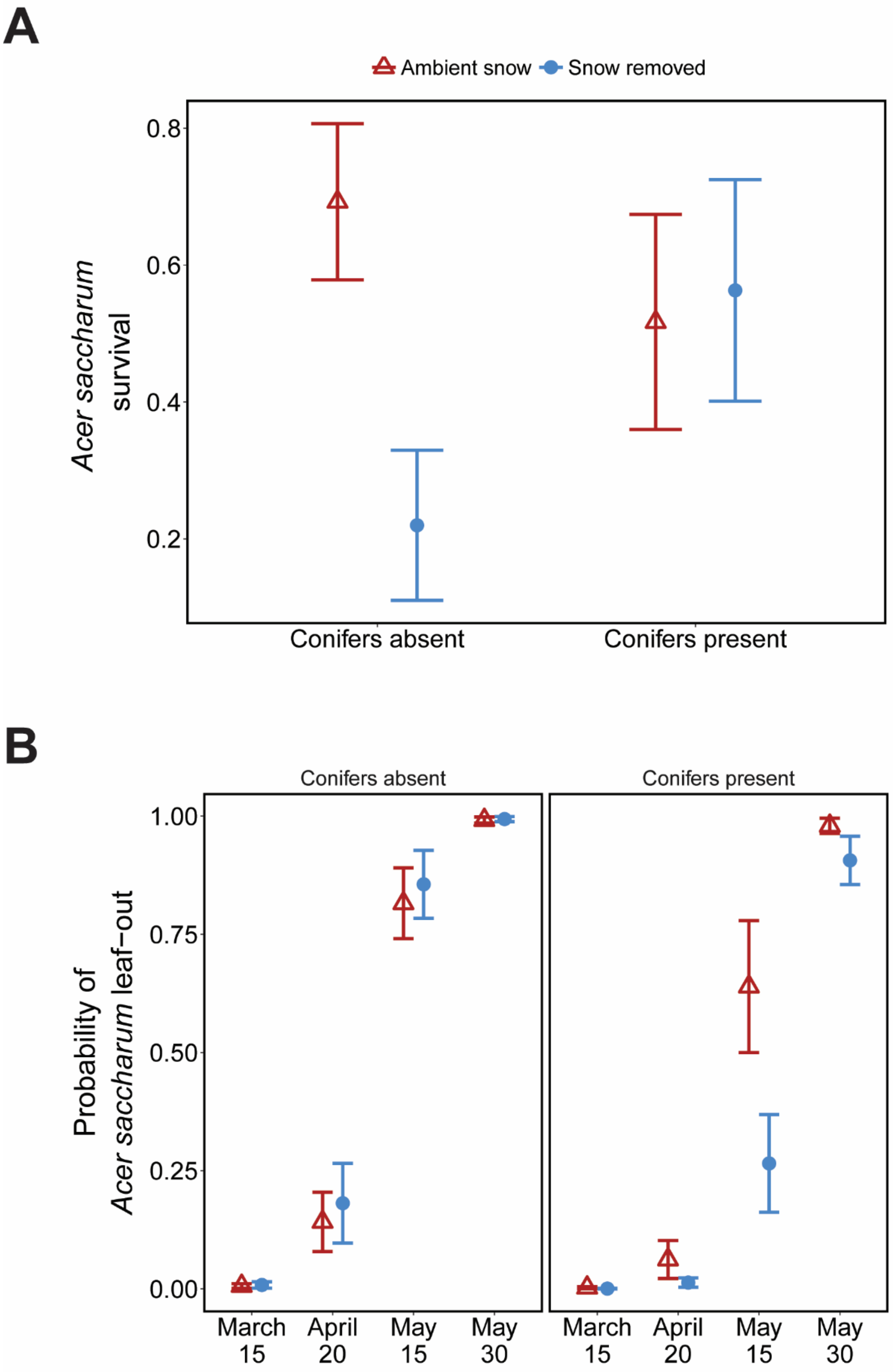

A
Ambient snow
Snow removed
Acer saccharum survival
0.2
0.4
0.6
0.8
Conifers absent
Conifers present
B
Conifers absent
Conifers present
Probability of Acer saccharum leaf-out
0.00
0.25
0.50
0.75
1.00
March 15
April 20
May 15
May 30
March 15
April 20
May 15
May 30

Figure 4