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ARTICLE
Vegetation Ecology

High sensitivity of herbaceous legumes to freezing: Insights from a multiyear snow removal study

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Abstract

Reductions in snow cover resulting from winter climate change are anticipated to increase the intensity of freezing exposure for overwintering herbaceous plants in many northern temperate regions. We examined whether herbaceous legumes, a key functional group responsible for increasing soil nitrogen inputs, are more sensitive to freezing than nonleguminous plants in northern temperate plant communities. We conducted snow removal experiments for three years in three herbaceous plant communities (a fallow area, an old field and a restored prairie) and recorded plant cover and biomass responses. For all three years, there were substantial decreases in legume biomass in response to snow removal for the fallow area (reductions of 70%, 90% and 50%; primarily *Melilotus albus* and *M. officinalis*) and old field (reductions of 50%, 60% and 50%; primarily *Trifolium pratense* and *Lotus corniculatus*), whereas there were no consistent significant reductions in biomass across years for the other herbaceous functional groups (graminoids and nonleguminous forbs). In contrast, in the restored prairie, dominated by the native legume *Desmodium canadense*, there were no consistent snow removal effects on the biomass of legumes or any other functional groups across years, although snow removal did reduce the percent cover of this species early in the growing season. Our results suggest that increased soil freezing via reductions in snow cover can have a stronger negative effect on some legumes than on other functional groups in northern temperate herbaceous plant communities, potentially reducing soil nitrogen inputs.

KEYWORDS
frost, nitrogen fixation, old field, snow cover, temperate, winter

INTRODUCTION

Freezing stress limits the productivity and distribution of many plant species (Yadav, 2010). In addition to increasing plant mortality, freezing can decrease germination (Croser et al., 2003) and damage overwintering or perennating structures (e.g., bulbs, taproots; Ambroise et al., 2020; Lubbe et al., 2021), leading to a host of sublethal effects (e.g., membrane damage, decreased N uptake, reduced biomass; Steponkus et al., 1993; Malyshev & Henry, 2012a). As a result, changes to freezing regimes can have pronounced impacts on plant communities and ecosystem processes (Kreyling, 2010; Smith & Sheridan, 2018). Snow cover can reduce exposure to freezing by providing a physical barrier

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between cold air temperatures and overwintering plants. Insulation by snow is of particular importance for herbaceous species because their overwintering structures are often positioned at or below the soil surface (Rapacz et al., 2014). In warm winters, although the length of the frozen season may be shortened, decreases in snow cover can increase the frequency and intensity of soil freeze–thaw cycles when freezing air temperatures do occur (e.g., overnight or during cold spells) (Henry, 2008). Likewise, increases in the variability of winter temperatures (Orsenigo et al., 2014; Vincent et al., 2018) and precipitation (Ahmed et al., 2022; Mekis & Vincent, 2011) can alter soil freezing dynamics; for example, both components can increase soil freeze–thaw cycling. Consequently, in the coming decades, the shoot bases, roots, and other belowground structures of overwintering plants will likely experience more frequent and severe freezing exposure in regions where snow cover is becoming more intermittent, but where periods of cold air temperatures persist (Christensen et al., 2007; Groffman et al., 2001; Henry, 2008; Rapacz et al., 2014).

Responses to freezing are not equal across taxa (e.g., Cici & Van Acker, 2011; Meyer & Badaruddin, 2001), and they can even vary within species (Farrell et al., 2006; Wisniewski et al., 2017) or between structures of an individual plant (Ambroise et al., 2020; Neuner, 2014). Understanding how different plant species respond to environmental variability is key to predicting potential shifts in community structure under future changes to environmental conditions over winter (Komac et al., 2015; Kreyling et al., 2012), including increased overwinter freezing exposure. Such changes may exert a strong influence on ecosystem function if specific plant functional groups are affected more than others. For example, legumes (Family: Fabaceae) can play a key functional role in nitrogen-limited ecosystems (e.g., Craine et al., 2002; Palmborg et al., 2005), given that most described legumes form mutualisms with diazotrophic (i.e., N-fixing) rhizobia (de Faria et al., 1989; Sprent et al., 2017). Legumes can increase soil fertility substantially (Lebauer & Treseder, 2008; Vitousek & Howarth, 1991) by increasing inputs of N-rich plant matter (Barker et al., 2022; Ritchie & Tilman, 1995; Zhao et al., 2015), carbon sequestration (via increased soil organic matter inputs) (De Deyn et al., 2011) and the complexity of soil faunal interactions (Gao et al., 2017; Zhao et al., 2014). Thus, environmental stressors that impact herbaceous legumes more severely than other herbaceous functional groups (e.g., graminoids and nonleguminous forbs) can have a substantial effect on ecosystems.

Among herbaceous plant species, emerging evidence suggests that legumes in northern temperate plant communities may be more susceptible to freezing than other herbaceous groups; for example, in response to increased soil freezing initiated by snow removal, legume cover decreased more than nonleguminous forb cover in a northern temperate old field (Lubbe & Henry, 2020). Similarly, in soil mesocosms exposed to increased spring freezing, the biomass of legumes was reduced substantially compared with both graminoid and nonleguminous forb biomass (Joseph & Henry, 2008). These examples were each based on the response of only a single legume species (red clover (*Trifolium pratense* L.) and crown vetch (*Securigera varia* L.) Lassen) in the respective studies. They therefore only provide anecdotal evidence that freezing sensitivity is higher in herbaceous legumes than in their neighboring species. Nevertheless, there also is a mechanistic basis underlying the potential for high freezing sensitivity in legumes; specifically, previous studies have reported decreased nodule formation in response to freezing (Alexandre & Oliveira, 2013; Thurston et al., 2022), and the resulting decrease in nitrogen fixation (Lorin et al., 2016) could reduce legume biomass production and reduce their competitive ability.

We conducted snow removal field experiments for three consecutive years, with plots targeted on patches where legumes were present, to assess the freezing tolerances of a range of herbaceous legumes relative to their nonleguminous neighbors. Our goal was to examine whether there would be consistency of findings across multiple plant communities, two dominated by non-native species and one dominated by native species, to provide a more comprehensive exploration of the freezing tolerances of legumes relative to other species than in previous studies. Following snow removal, we examined percent cover and biomass for each species over the subsequent growing season, and we established a new set of snow removal plots each year. Based on the prior observations of herbaceous legumes being more sensitive to freezing relative to other herbaceous species, we predicted the herbaceous legumes would exhibit greater reductions in cover and biomass in response to snow removal than their nonleguminous neighbors.

METHODS

Study site

The study was conducted within three herbaceous plant communities at the Environmental Sciences Western (ESW) field station near Ilderton, Ontario, Canada (43°04′29.2″ N, 81°20′16.0″ W). The plant communities, (1) an old field (left to naturalize following the abandonment of cropland), (2) a restored tallgrass prairie (on former cropland), and (3) a fallow area (adjacent to active agricultural plots), were selected

based on the presence of established herbaceous legume populations representing a variety of species. None of the three sites were managed, with the exception of a prescribed burn that was conducted in the prairie two years prior to the beginning of the experiment. Legume species investigated at each site were as follows: old field - red clover (*Trifolium pratense* L.) and bird's-foot trefoil (*Lotus corniculatus* L.); tallgrass prairie - showy tick-trefoil (*Desmodium canadense* (L.) DC.), which was the sole native target legume species in the study; and fallow - white sweet clover (*Melilotus albus* Medik.) and yellow sweet clover (*Melilotus officinalis* (L.) Pall.), with *Melilotus* spp. pooled for analysis due to the high interannual variation in the abundance of each species. The dominant species in the old field were goldenrod (*Solidago* spp.), common dandelion (*Taraxacum officinale* Weber), and red clover (*Trifolium pratense*); other abundant species included thistles (*Cirsium* spp. and *Sonchus* spp.), American asters (*Symphyotrichum* spp.), avens (*Geum* spp.), wild carrot (*Daucus carota* L.), and birds-foot trefoil (*Lotus corniculatus*). The only consistently dominant species in the tallgrass prairie was goldenrod (*Solidago* spp.); several other plant species that were abundant in patches included wild bergamot (*Monarda fistulosa* L.), grayhead coneflower (*Ratibida pinnata* (Vent.) Barnhart), American asters (*Symphyotrichum* spp.), rosinweeds (*Silphium* spp.), sedges (*Carex* spp.), showy tick-trefoil (*Desmodium canadense*), native C4 grasses (e.g., *Andropogon gerardii* Vitman), and other coneflowers (*Rudbeckia* spp.). Within the fallow area, the dominant species were goldenrod (*Solidago* spp.) and common dandelion (*Taraxacum officinale*); other abundant species included non-native grasses (e.g., *Bromus inermis* Leyss., *Poa compressa* L.), Virginia strawberry (*Fragaria virginiana* Mill.), black medic (*Medicago lupulina* L.), wild carrot (*Daucus carota*), oxeye daisy (*Leucanthemum vulgare* Lam.), fleabanes (*Erigeron annuus* (L.) Pers. and *E. philadelphicus* L.), and common St. John's wort (*Hypericum perforatum* L.). The relative abundances of all plant species (based on percentage of total cover) in the control plots for each of the three plant communities are displayed in Rycroft (2023). The soil was characterized as London clay loam in the fallow area, and Bryanston silt loam in the old field and restored prairie (Hagerty & Kingston, 1992; detailed soil properties are characterized in Stover & Henry, 2020).

Snow removal

Within each site, ten pairs of 1 m × 1 m plots (20 plots per site; 60 plots total), where the target leguminous species were present at a density of at least three individual plants per square meter, were marked on 23–24 October 2018 (mean total stem densities for the forbs were 146 and 138 m^{-2} for the old field and prairie, respectively; Rycroft, 2023). Plots were paired based on proximity and similarity of vegetation and randomly assigned therein to either a snow removal (via shoveling) or control (ambient snow) treatment; snow removal was used to increase soil freezing relative to the control. Snow removal ceased in early March to minimize any potential effects of snow removal on end-of-season snow melt and subsequent effects on soil moisture during the growing season; in each of the study years, there was consistent snow cover on the ambient snow and snow removal plots prior to and during spring melt. Soil temperature loggers (LogTag TRIX-8, MicroDAQ, NH, USA) were placed 2 cm beneath the soil surface within a subset of both the snow removal and control plots at each site ($n = 3$ per treatment within the prairie and old field sites; $n = 3$ for snow removal and $n = 2$ for control plots within the fallow site) to measure soil temperatures hourly.

All plots were covered with Winter Wrap (Quest Plastics)—a white plastic netting (1 cm mesh size)—on 8 November 2018 (old field and fallow) and 15 November 2018 (prairie) prior to treatment application to delineate the appropriate snow removal depth and prevent removal of surface litter or structures and subsequent damage during snow removal. Netting was affixed over the surface of the litter using ground staples. Snow removal was then conducted when adequate snow accumulation (≥5 cm) coincided with air temperatures sufficiently cold to increase soil freezing (≤−10°C); in the first year, snow removal was conducted nine times from 21 November 2018 through 6 March 2019. Soil temperature loggers and Winter Wrap were removed on 6 April 2019 to prevent any impacts on aboveground plant growth within the plots. In the second year, ten new pairs of 1 m × 1 m plots per site (20 plots per site; 60 plots total) were established on 28 October 2019 following the same criteria and setup outlined above. Winter Wrap was affixed to plots on 8 December 2019 (fallow and old field) and on 17 December 2019 (prairie). Snow removal was conducted a total of seven times between 20 January 2020 and 28 February 2020. The plastic netting and soil temperature loggers were removed on 3 April 2020. In the third year, 10 new pairs of plots were again marked within each site (20 plots per site; 60 plots total) on 17 October 2020, following the same criteria and setup as the first two years. Plastic netting was applied to all plots on 28–29 November 2020, and snow removal was conducted a total of eight times between 7 January 2021 and 12 February 2021. Soil temperature loggers and plastic netting were removed from the plots on 23 April 2021.

Data collection

Plant cover for each plot was assessed through visual surveys in the growing season following treatment application, with multiple surveys conducted each year to assess different periods of the growing season and to account for variation in phenology among species. Surveys following the first year of snow removal were conducted on 3–7 June 2019, 13–15 July 2019, and 7–10 August 2019. For the second year, surveys were conducted June 9–14 2020, 16–19 July 2020, and 15–18 August 2020. In the final year, surveys were conducted 10–13 June 2021, 16–18 July 2021, and 14–18 August 2021.

Aboveground plant material for each plot was harvested at the peak of the growing season (August–September, dependent on site) by using an electric hedge trimmer to sample from a 60 cm × 40 cm section of each plot. The aboveground plant material was then sorted by plot into functional groups: nonleguminous forbs (NLF), leguminous forbs (LF), and graminoids (GR); within LF, plant material was further separated to the species level. The plant matter was subsequently dried at 60°C for at least 72 h or until mass measurements became constant. Harvest in the first year was carried out on 8–9 August 2019 (fallow and old field) and 6 September 2019 (prairie). For subsequent years, harvests were conducted on 16 August 2020 for fallow plots, 17 August 2020 for old field plots, and 9–10 September 2020 for prairie plots, and 16 August 2021 for the fallow plots, 16–17 August 2021 for plots within the old field site, and 1–4 September 2021 for the prairie site. We did not examine the impacts of multiple years of snow removal within a given plot because the destructive biomass sampling necessitated that new plots had to be selected each year to ensure a sufficient abundance of target legumes was present.

Data analyses

To examine treatment effects on biomass, a mixed-effects model with year, functional group, and treatment (snow removal versus control) as fixed effects and plot pair as a random effect was used for each site; linear contrasts were then used to directly compare whether treatment-induced changes in legume biomass differed significantly from the changes exhibited by the other functional groups. In addition, the responses of total, functional group, and legume (species-level) aboveground biomass to snow removal were analyzed using paired one-tailed t tests within each plant community for each year. Snow removal effects on species-level cover were assessed for each year using paired one-tailed t tests for each survey period; species-level cover was analyzed only for the dominant species (i.e., those in all or nearly all plots) and target legumes within each plant community. All analyses were conducted using JMP Version 14 (1989–2023) (SAS Institute).

RESULTS

Interannual variation in weather and treatment effects on soil temperature and freeze–thaw

Over the three years, there were interannual differences in both winter air temperatures and snow cover. The first winter (December–March) was the coldest (average daily temperature −3.2°C) with regular snow cover (2.8 cm on average) (Environment Canada, 2022). The second winter was extremely mild (average daily temperature −0.3°C) with lower-than-normal snow cover (2.2 cm on average), but a high proportion of snow near the end of the snow removal period. The winter in the final year was also mild (average daily temperature −1.5°C; although it was severely cold during periods of snow removal), with low snow cover (0.9 cm on average), especially in the latter portion of the winter.

The effect of snow removal on average soil temperature, minimum temperature, and number of freeze–thaw cycle events varied by year (Figure 1). Nevertheless, snow removal decreased the minimum soil temperature across all sites relative to the control (ambient snow) in all years (Figure 1A). Snow removal typically increased the number of freeze–thaw events, except for the fallow area and old field in the first year (Figure 1C).

Aboveground biomass and cover responses

For the fallow site, the mixed-effects model results revealed there was a significant interaction between treatment effect (snow removal versus control) and functional group ($p_{\text{Functional×Treatment}} = 0.013$). The aboveground biomass of leguminous forbs was reduced substantially by snow removal in all years, with approximate reductions relative to the control plots of 70% in Year 1 ($p < 0.05$), 90% in Year 2 ($p < 0.01$) and 50% in Year 3 ($p < 0.001$) (Figure 2A). These decreases were explained almost entirely by declines in *Melilotus* spp. in response to snow removal (Figure 2B). In contrast, there were no significant effects of snow removal on the aboveground biomasses of graminoids or nonleguminous forbs, except for in Year 3, when nonleguminous forb biomass was reduced by ~12% ($p < 0.001$; Figure 2A). The linear contrasts confirmed there was a larger reduction in biomass caused by snow removal in legumes than in graminoids ($p_{\text{GRvsLF}} = 0.0041$), and there was a larger

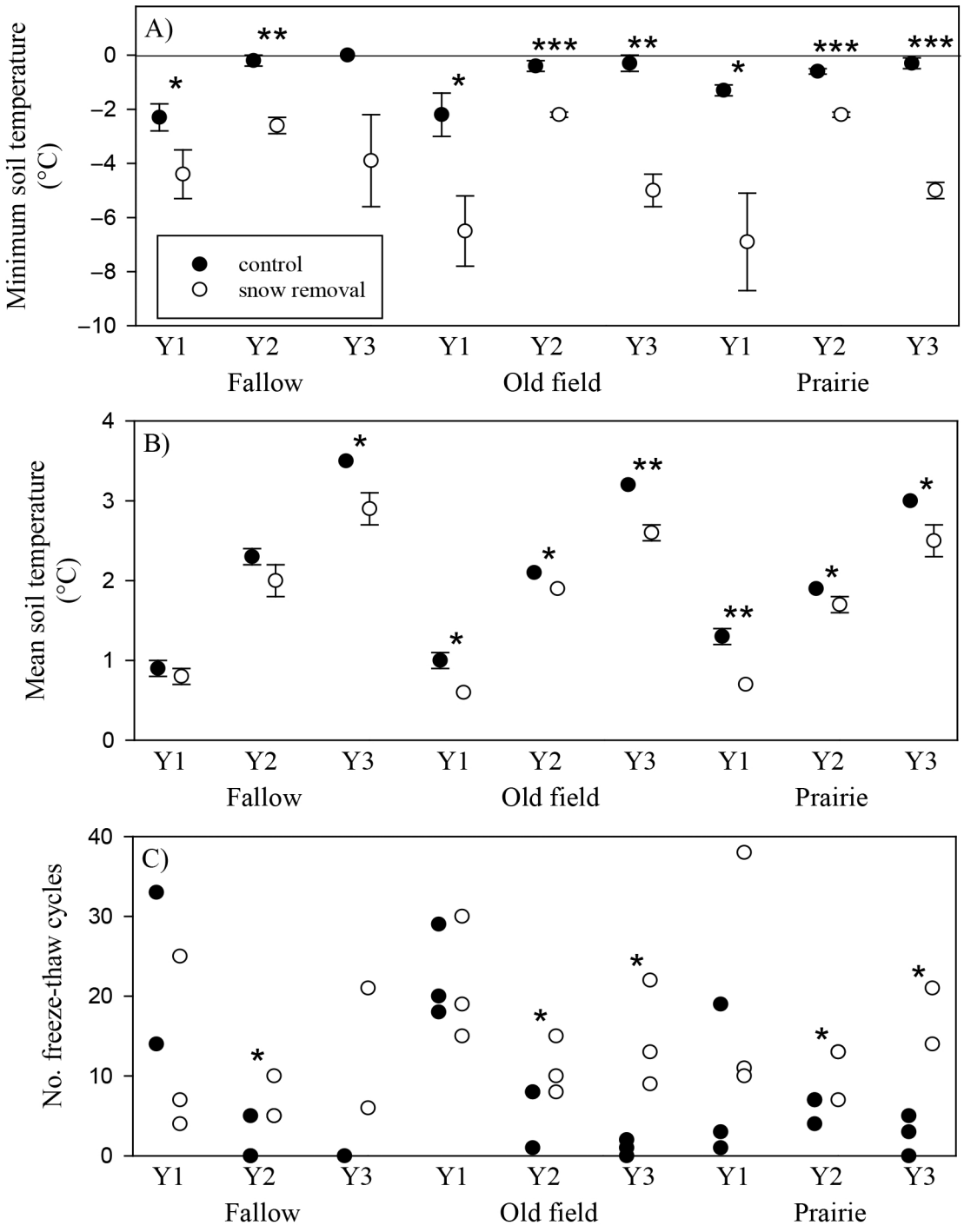


FIGURE 1 (A) Minimum winter soil temperature at 2 cm depth (mean ± SE), (B) mean soil temperature at 2 cm depth (mean ± SE), and (C) the number of freeze–thaw cycles per plot (defined as decreases in temperature below 0°C followed by an increase in temperature above 0°C) in years 1, 2, and 3 (Y1, Y2, and Y3) for the fallow area, old field, and prairie (December–April). Asterisks indicate significant differences (*$0.01 < p < 0.05$; **$0.001 < p < 0.01$; ***$p < 0.001$; paired one-tailed t test) between treatments for a given site and year of study.

reduction in biomass caused by snow removal in legumes than in nonleguminous forbs ($p_{\text{NLFvsLF}} = 0.039$).

For the old field, the mixed-model analysis revealed that biomass responses to the snow removal treatment depended on functional group ($p_{\text{Functional×Treatment}} = 0.0002$). The aboveground biomass of leguminous forbs was reduced substantially by snow removal in all years, with approximate reductions relative to the control plots of 50% in Year 1 ($p < 0.01$), 60% in Year 2 ($p < 0.001$) and 50% in Year 3 ($p < 0.001$) (Figure 3A). These declines were predominantly explained by reductions in *T. pratense* and *L. corniculatus* (Figure 3B). Similar to the fallow site, there

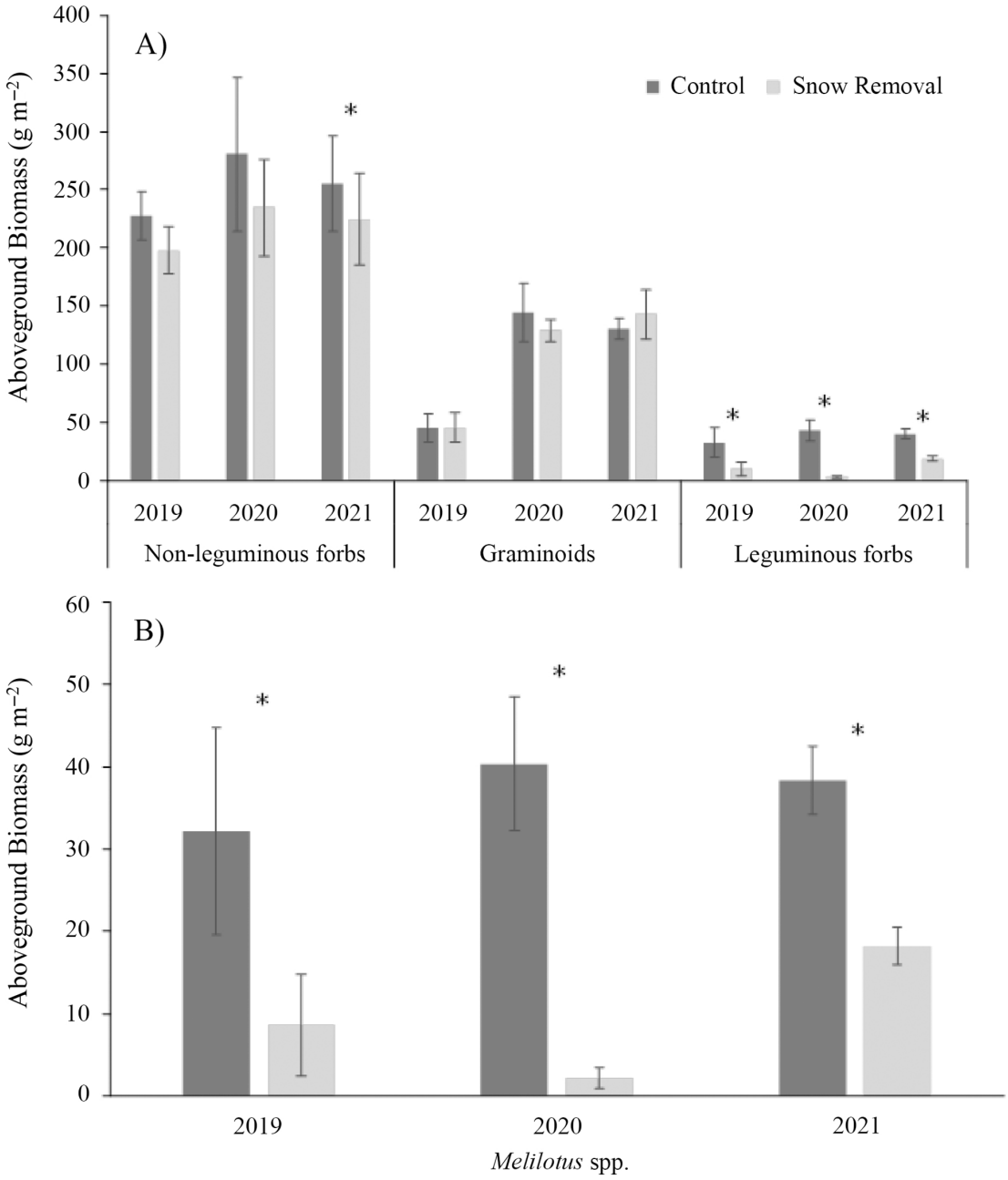


FIGURE 2 Dried aboveground biomass (mean ± SE) harvested at peak growth of (A) herbaceous functional groups and (B) the target herbaceous legumes, *Melilotus* spp. under snow removal and control treatments across three years of experimentation within the fallow site ($n = 10$ per treatment). Asterisks (*) indicate significant differences ($p < 0.05$; paired one-tailed t test) between treatments for a given species or functional group within each year of study.

were no significant effects of snow removal on the aboveground biomasses of graminoids or nonleguminous forbs, except for Year 2, when nonleguminous forb biomass was reduced by ~19% ($p < 0.01$; Figure 3A). The linear contrasts confirmed there was a larger reduction in biomass caused by snow removal in legumes than in graminoids ($p_{\text{GRvsLF}} = 0.0001$), and there was a larger reduction in biomass caused by snow removal in legumes than in nonleguminous forbs ($p_{\text{NLFvsLF}} = 0.001$).

For the prairie site, the mixed-model analysis of biomass revealed a marginally significant interaction between treatment and functional group ($p_{\text{Functional×Treatment}} = 0.051$). However, unlike in the fallow site or old field, the aboveground biomass of leguminous forbs was not affected significantly by snow removal in any year (Figure 4A). This response was explained by the lack of response by the dominant legume species, *Desmodium canadense* (Figure 4B). There were no significant reductions in aboveground

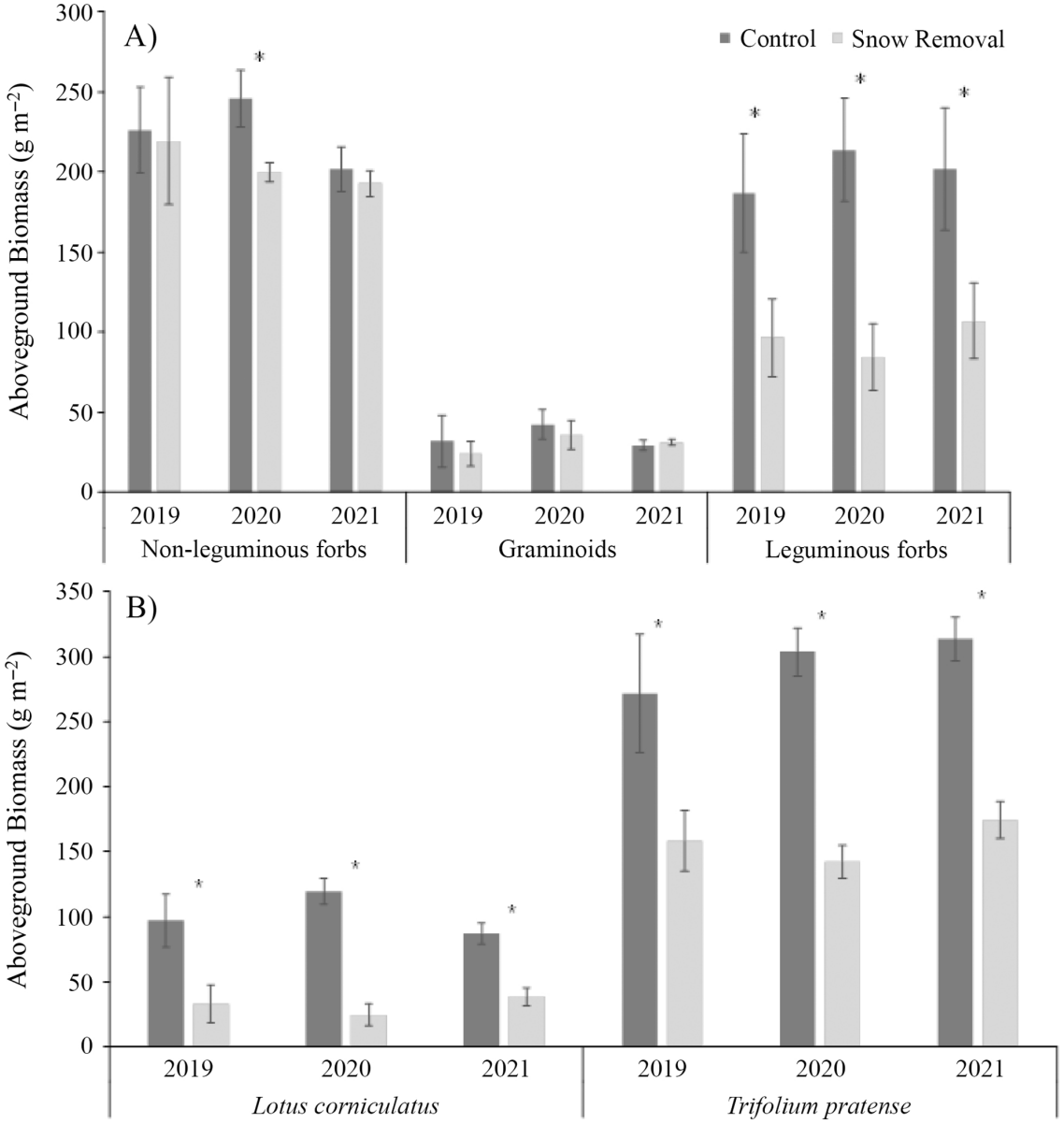


FIGURE 3 Dried aboveground biomass (mean ± SE) harvested at peak growth of (A) herbaceous functional groups ($n = 10$ per treatment) and (B) the target herbaceous legumes, *Lotus corniculatus* and *Trifolium pratense* ($n = 5$ per treatment per species) under snow removal and control treatments across three years of experimentation within the old field site. Asterisks (*) indicate significant differences ($p < 0.05$; paired one-tailed t test) between treatments for a given species or functional group within each year of study.

biomass in response to snow removal for the graminoids or nonleguminous forbs, except for in Year 1, when graminoid biomass was reduced by ~80% ($p < 0.05$; Figure 4A). The linear contrasts revealed there was not a larger reduction in biomass caused by snow removal in legumes than in graminoids ($p_{GRvsLF} = 0.34$), nor was there a larger reduction in biomass caused by snow removal in legumes than in nonleguminous forbs ($p_{NLFvsLF} = 0.14$).

In general, the treatment effects on plant cover (Tables 1–3) examined over the growing season were consistent with the aboveground biomass responses. However, the prairie legume *Desmodium canadense*, which did not exhibit significant biomass responses to snow removal, exhibited significant reductions in cover early in the growing season in response to snow removal in Year 1 and Year 2 (Table 3).

DISCUSSION

While various factors affecting legume freezing tolerance (e.g., cultivar, exogenous phytohormone application, nutrient provision, freezing timing and severity; Bertrand et al., 2007; D'Amours et al., 2022; Irshad et al., 2021;

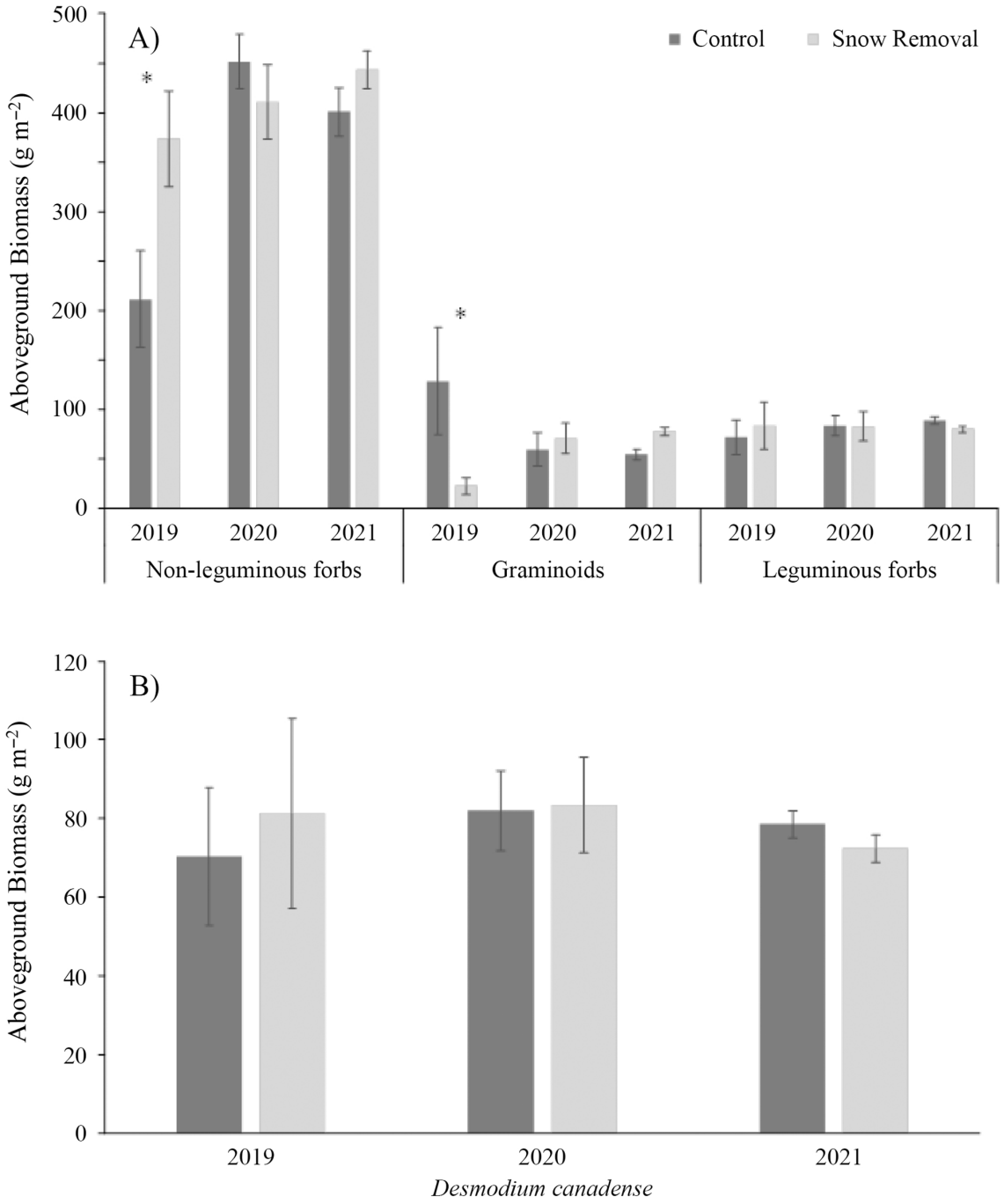


FIGURE 4 Dried aboveground biomass (mean ± SE) harvested at peak growth of (A) herbaceous functional groups and (B) the target herbaceous legume, *Desmodium canadense*, under snow removal and control treatments across three years of experimentation within the prairie site ($n = 10$ per treatment). Asterisks (*) indicate significant differences ($p < 0.05$; paired one-tailed t test) between treatments for a given species or functional group within each year of study.

McKersie et al., 1993, 1999; Mir et al., 2021) have been studied extensively in an agricultural context, where legumes are often planted in monoculture, the freezing tolerances of legumes relative to other species in herbaceous plant communities have not been a focus of previous studies. Our prediction that herbaceous legumes would be more sensitive to freezing than neighboring nonleguminous herbaceous plants was supported for the two sites that were dominated by non-native legumes, but the response of *Desmodium canadense* (the sole dominant native herbaceous legume) at the prairie site was contrary to our prediction, at least for the peak growing season biomass results. The non-native legumes consisted of naturalized populations that have escaped cultivation

TABLE 1 Percent cover (mean ± SE) for the target herbaceous legumes (*Melilotus albus* and *M. officinalis*, pooled) and dominant herbaceous species within the control and snow removal (SR) plots ($n = 10$ per treatment) at the fallow site over the three years.

	June		July		August	
Species	**Control**	**SR**	**Control**	**SR**	**Control**	**SR**
Year 1						
Melilotus spp.	9 ± 2	2 ± 1**	11 ± 1	4 ± 1***	11 ± 1	4 ± 1***
Taraxacum officinale	5 ± 1	6 ± 1	2 ± 1	3 ± 1	3 ± 1	4 ± 1
Solidago spp.	37 ± 6	45 ± 6	41 ± 7	45 ± 7	42 ± 7	45 ± 7
Year 2						
Melilotus spp.	1 ± 0	0 ± 0*	4 ± 1	0 ± 0***	5 ± 1	2 ± 1***
Taraxacum officinale	7 ± 1	7 ± 1	5 ± 1	7 ± 1	7 ± 1	8 ± 1
Solidago spp.	36 ± 6	39 ± 7	35 ± 6	33 ± 6	35 ± 6	33 ± 6
Year 3						
Melilotus spp.	2 ± 1	0 ± 0*	3 ± 1	0 ± 0**	4 ± 1	2 ± 1**
Taraxacum officinale	7 ± 2	7 ± 1	8 ± 1	10 ± 2	9 ± 1	12 ± 1*
Solidago spp.	31 ± 5	29 ± 3	36 ± 4	32 ± 3	39 ± 5	35 ± 3

Note: Asterisks indicate significant differences ($p < 0.05$, paired one-tailed t test) between treatments within a species for a given year.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 2 Percent cover (mean ± SE) for the target herbaceous legumes (*Lotus corniculatus* and *Trifolium pratense*) and dominant herbaceous species within control and snow removal (SR) plots ($n = 10$ per treatment) at the old field site over the three years.

	June		July		August	
Species	**Control**	**SR**	**Control**	**SR**	**Control**	**SR**
Year 1						
Lotus corniculatus	17 ± 3	7 ± 2*	28 ± 5	18 ± 3*	24 ± 4	14 ± 2*
Trifolium pratense	44 ± 6	25 ± 3*	68 ± 5	42 ± 10*	56 ± 3	34 ± 6**
Taraxacum officinale	9 ± 1	13 ± 2	3 ± 1	3 ± 1	8 ± 1	9 ± 1
Solidago spp.	21 ± 2	29 ± 4*	27 ± 6	43 ± 6*	33 ± 5	40 ± 5
Year 2						
Lotus corniculatus	31 ± 6	20 ± 5**	30 ± 5	18 ± 4**	32 ± 4	17 ± 2**
Trifolium pratense	32 ± 4	21 ± 3**	30 ± 4	19 ± 3**	33 ± 3	17 ± 2**
Taraxacum officinale	15 ± 1	14 ± 1	12 ± 1	14 ± 2	15 ± 2	12 ± 2
Solidago spp.	25 ± 2	20 ± 3*	27 ± 2	19 ± 2*	24 ± 1	23 ± 2
Year 3						
Lotus corniculatus	21 ± 2	9 ± 2**	26 ± 2	15 ± 2***	28 ± 3	18 ± 2**
Trifolium pratense	27 ± 3	15 ± 2**	31 ± 3	18 ± 3**	37 ± 5	20 ± 2*
Taraxacum officinale	14 ± 2	13 ± 1	10 ± 1	10 ± 1	12 ± 2	12 ± 1
Solidago spp.	25 ± 3	23 ± 2	27 ± 2	26 ± 2	29 ± 2	26 ± 1

Note: Cover for target legumes was averaged within target plots ($n = 5$ per treatment). Asterisks indicate significant differences ($p < 0.05$, paired one-tailed t test) between treatments within a species for a given year.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

following their introduction for agricultural and other purposes (e.g., as a preventative measure for soil erosion; Turkington & Franko, 1980). Therefore, the source populations may have originated (i.e., via commercial seed suppliers) from southern climates that experience milder winters, and thus may not be as well adapted as their neighboring species to harsher winters. Similarly, the freezing sensitive legumes in previous studies were

TABLE 3 Percent cover (mean ± SE) for the target herbaceous legume (*Desmodium canadense*) and dominant herbaceous species within control and snow removal (SR) plots ($n = 10$ per treatment) at the prairie site over the three years.

	June		July		August	
Species	**Control**	**SR**	**Control**	**SR**	**Control**	**SR**
Year 1						
Desmodium canadense	13 ± 2	9 ± 2*	15 ± 1	11 ± 3	14 ± 1	12 ± 1
Solidago spp.	17 ± 5	24 ± 8*	24 ± 6	25 ± 7	25 ± 6	26 ± 8
Year 2						
Desmodium canadense	25 ± 2	13 ± 2***	29 ± 2	18 ± 2***	26 ± 1	23 ± 2
Solidago spp.	13 ± 3	10 ± 3	13 ± 3	11 ± 3	14 ± 2	13 ± 3
Year 3						
Desmodium canadense	15 ± 1	12 ± 1	16 ± 1	14 ± 1	17 ± 1	16 ± 1
Solidago spp.	11 ± 2	12 ± 2	14 ± 2	16 ± 2	15 ± 1	19 ± 2*

Note: Asterisks indicate significant differences ($p < 0.05$, paired one-tailed t test) between treatments within a species for a given year.
*$p < 0.05$; ***$p < 0.001$.

non-native species (e.g., Joseph & Henry, 2008; Lubbe & Henry, 2020). Nevertheless, although the biomass of *Desmodium canadense*, the native legume, was not sensitive to freezing, the early season coverage of this species decreased with snow removal, possibly from damage to overwintering structures. Moreover, freezing experiments on both potted and transplanted legumes have revealed that low legume freezing tolerance relative to other species in northern temperate regions is not unique to non-native legumes (Rycroft & Henry, 2024). The apparent recovery of *D. canadense* within a single growing season potentially could be explained by a reallocation of resources from reproductive (i.e., seed production) to vegetative growth, as has occurred for previous freezing experiments on the grass *Poa pratensis* (Malyshev & Henry, 2012b).

We based our prediction of low legume freeze tolerance in part on the observation that decreased nodule formation in response to freezing (Alexandre & Oliveira, 2013; Thurston et al., 2022) provides a mechanistic explanation for high freezing sensitivity in legumes. Such a mechanism implies that the presence of compatible, freeze-tolerant rhizobia may be an important component of legume freeze tolerance. Numerous studies have demonstrated the benefits of rhizobial associations under a myriad of stressors, including freezing and low temperature stress (e.g., Liu et al., 2019; Prévost et al., 1999, 2003). For example, alfalfa in association with cold-adapted rhizobia had significantly improved regrowth following overwintering under both anaerobic winter conditions (i.e., simulated ice encasement; ~40% more dry shoot mass) and normal winter conditions (~20% greater dry shoot mass; Prévost et al., 2003). As the sites we studied were previously used for agriculture, the soils at these sites may be degraded with respect to the diversity of soil organisms (Middleton & Bever, 2012), especially bacterial diversity (Martínez-Romero & Caballero-Mellado, 1996), compared to remnant or relatively undisturbed habitats. Therefore, the soils of these sites may contain nonoptimal rhizobia or lack compatible rhizobia for these species, which can impair nitrogen fixation (Denison & Kiers, 2004; Kiers & Denison, 2008).

Root structure also may have explained the high freezing sensitivities of the non-native legumes. The belowground structure of these legumes, particularly *Trifolium pratense* and *Melilotus officinalis*, consists of a pronounced primary taproot (Rupert 1989; Chmelíková & Hejcman, 2012). These species, as with other tap-rooted leguminous species (e.g., alfalfa; Perfect et al., 1987) and even nonlegumes, may be vulnerable to frost heave, wherein patchy freezing of soil water occurs, causing expansion and subsequent upheaval of soil and proximal, susceptible plant structures located at or near the soil surface (Iwata et al., 1995). While tap-rooted plants can be thrust out of the soil by frost heave, leading to either lethal or sublethal damage (Perfect et al., 1988), more fibrous root systems may be better anchored and less disrupted (e.g., Jonasson, 1986). Although true fibrous root systems would have been restricted to the graminoid species in our study, unlike *Trifolium pratense* and *Melilotus officinalis* (Chmelíková & Hejcman, 2012), *Desmodium canadense* features a robust lateral root system and a relatively deep root collar depth, both of which could render it less vulnerable to frost heave than the non-native legume species. Under significant frost heaving, fine roots growing off the taproot (which are key for nutrient acquisition; Ambroise et al., 2020; Tierney et al., 2001) and nodules (which house rhizobial partners and are thus essential for nitrogen fixation) can become exposed to more severe freezing temperatures, resulting in subsequent damage or

mortality (Matamoros et al., 1999; Tierney et al., 2001). Damage to these structures may then lead to resources (e.g., nonstructural carbohydrates; Gaul et al., 2008) being allocated to repair or regrow vital components, potentially placing a resource strain on the affected plant. Concentrations of various nutrients (e.g., N, K, and phytate) and proteins increase during winter hardening within the taproots of some herbaceous legumes (i.e., the taproots function as storage organs), and they decrease significantly in spring prior to the initiation of regrowth in roots of birds-foot trefoil, red clover, and sweet clover (Li et al., 1996); accumulations of nonstructural carbohydrates and other nutrients/compounds have been hypothesized to allow for greater regrowth in the early growing season. If belowground tissues (e.g., fine roots, primary taproot) containing high concentrations of these nutrients or proteins are damaged significantly or die due to increased freezing exposure during overwintering, this may exert an additional resource-based strain on the affected legumes and in part explain the lowered growth seen in situ. Damage to fine roots exposed to more extreme freezing temperatures following frost heave may also lead to water deficits, impacting growth the following summer (Zahran, 1999; Marino et al., 2007; González et al., 2015; Ambroise et al., 2020).

While our snow removal treatment was intended to increase the exposure of overwintering plants to freezing, rather than to directly simulate the conditions experienced by plants in warmer winters, increased mid-winter melts caused by increased climate variability (Vincent et al., 2018), changes in winter precipitation (Ahmed et al., 2022) and increases in mean winter temperature (Henry, 2008) all result in the exposure of overwintering plants to an increased frequency and intensity of freeze–thaw cycles in the context of climate change in northern temperate regions (Groffman et al., 2001). Given that snow removal can potentially affect plant growth via changes to soil moisture over the subsequent growing season, we ceased snow removal prior to the end of winter to avoid confounding the effects of changes to both freezing intensity and soil moisture. Consequently, in each year, there was consistent snow cover on the ambient snow and snow removal plots during spring melt. Similarly, for previous snow removal studies at our site and others where this technique was used, there were no significant treatment effects on soil moisture in spring (Vankoughnett & Henry, 2014; Henry et al., 2018; Stover & Henry, 2019). However, ceasing snow removal in late winter also meant that our experiment did not address the potential effects of spring freezing. The freezing sensitivities of legumes relative to other species may be particularly high in the spring (e.g., Joseph & Henry, 2008), which suggests that variation in the timing of cold deacclimation could also influence legume freezing responses beyond what we observed in our study. Reductions in snow cover can increase soil warming by increasing the sun exposure of the soil surface and decreasing albedo, which can affect the phenology of many herbaceous species by advancing growth and development (Vankoughnett et al., 2016; Sherwood et al., 2017). Premature cold deacclimation can increase the susceptibility of structures at or near the soil surface to subsequent periods of freezing (Bokhorst et al., 2008, 2009, 2011; Vyse et al., 2019), which suggests that increased spring temperature variability could pose further challenges for some species.

Conclusions

Our findings suggest that herbaceous legumes may be highly impacted by increased freezing exposure in the coming decades relative to nonlegumes. Although our study examined the effects of increased freezing exposure over multiple years, allowing for insights into plant responses in the context of interannual variation in winter conditions (e.g., snow cover, temperatures, freeze–thaw cycling), we did not examine the impacts of multiple years of snow removal within a given plot. However, multiple consecutive years of increased soil freezing could potentially elicit stronger responses. Legacy effect studies would also be useful to demonstrate the extent to which reductions in legumes in response to freezing in one year might carry over to subsequent years. If these effects persist, severe freezing events could have the lasting effect of reduced nitrogen inputs into these systems. Beyond the study of restored and naturalized plant communities, our results may have broader implications for agriculture, such as for the overwintering success of fall-planted legumes in cover crop mixtures.

AUTHOR CONTRIBUTIONS

Both Samuel L. Rycroft and Hugh A. L. Henry designed the study, analyzed the data, and wrote the paper. Samuel L. Rycroft performed the research.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Rycroft & Henry, 2025) are available from Dryad: https://doi.org/10.5061/dryad.xwdbrv1nw.

REFERENCES

Ahmed, S. I., R. Rudra, P. Goel, A. Amili, T. Dickinson, K. Singh, and A. Khan. 2022. "Change in Winter Precipitation Regime Across Ontario, Canada." *Hydrology* 9(5): 81. https://doi.org/10.3390/hydrology9050081.

Alexandre, A., and S. Oliveira. 2013. "Response to Temperature Stress in Rhizobia." *Critical Reviews in Microbiology* 39: 219–228. https://doi.org/10.3109/1040841X.2012.702097.

Ambroise, V., S. Legay, G. Guerriero, J. F. Hausman, A. Cuypers, and K. Sergeant. 2020. "The Roots of Plant Frost Hardiness and Tolerance." *Plant and Cell Physiology* 61: 3–20. https://doi.org/10.1093/pcp/pcz196.

Barker, W., L. S. Comita, S. J. Wright, O. L. Phillips, B. L. Sedio, and S. A. Batterman. 2022. "Widespread Herbivory Cost in Tropical Nitrogen-Fixing Tree Species." *Nature* 612: 483–87. https://doi.org/10.1038/s41586-022-05502-6.

Bertrand, A., D. Prévost, F. J. Bigras, and Y. Castonguay. 2007. "Elevated Atmospheric CO_2 and Strain of *Rhizobium* Alter Freezing Tolerance and Cold-Induced Molecular Changes in Alfalfa (*Medicago sativa*)." *Annals of Botany* 99: 275–284.

Bokhorst, S. F., J. W. Bjerke, F. W. Bowles, J. Melillo, T. V. Callaghan, and G. K. Phoenix. 2008. "Impacts of Extreme Winter Warming in the Sub-Arctic: Growing Season Responses of Dwarf Shrub Heathland." *Global Change Biology* 14: 2603–12. https://doi.org/10.1111/j.1365-2486.2008.01689.x.

Bokhorst, S. F., J. W. Bjerke, L. E. Street, T. V. Callaghan, and G. K. Phoenix. 2011. "Impacts of Multiple Extreme Winter Warming Events on Sub-Arctic Heathland: Phenology, Reproduction, Growth, and CO_2 Flux Responses." *Global Change Biology* 17: 2817–30. https://doi.org/10.1111/j.1365-2486.2011.02424.x.

Bokhorst, S. F., J. W. Bjerke, H. Tømmervik, T. V. Callaghan, and G. K. Phoenix. 2009. "Winter Warming Events Damage Sub-Arctic Vegetation: Consistent Evidence from an Experimental Manipulation and a Natural Event." *Journal of Ecology* 97: 1408–15. https://doi.org/10.1111/j.1365-2745.2009.01554.x.

Chmelíková, L., and M. Hejcman. 2012. "Root System Variability in Common Legumes in Central Europe." *Biologia* 67: 116–125. https://doi.org/10.2478/s11756-011-0138-7.

Christensen, J. H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, et al. 2007. "Regional climate projections." In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, 847–940. Cambridge, UK: Cambridge University Press.

Cici, S. Z. H., and R. C. Van Acker. 2011. "Relative Freezing Tolerance of Facultative Winter Annual Weeds." *Canadian Journal of Plant Science* 91: 759–763. https://doi.org/10.4141/cjps2010-001.

Craine, J. M., D. Tilman, D. Wedin, P. Reich, M. Tjoelker, and J. Knops. 2002. "Functional Traits, Productivity and Effects on Nitrogen Cycling of 33 Grassland Species." *Functional Ecology* 16: 563–574. https://doi.org/10.1046/j.1365-2435.2002.00660.x.

Croser, J. S., H. J. Clarke, K. H. M. Siddique, and T. N. Khan. 2003. "Low-Temperature Stress: Implications for Chickpea (*Cicer arietinum* L.) Improvement." *Critical Reviews in Plant Sciences* 22: 185–219. https://doi.org/10.1080/713610855.

D'Amours, E., A. Bertrand, J. Cloutier, A. Claessens, S. Rocher, and P. Seguin. 2022. "Impact of *Sinorhizobium meliloti* Strains and Plant Population on Regrowth and Nodule Regeneration of Alfalfa after a Freezing Event." *Plant and Soil* 500(1-2): 161–179. https://doi.org/10.1007/s11104-022-05662-4.

De Deyn, G. B., R. S. Shiel, N. J. Ostle, N. P. McNamara, S. Oakley, I. Young, C. Freeman, N. Fenner, H. Quirk, and R. D. Bardgett. 2011. "Additional Carbon Sequestration Benefits of Grassland Diversity Restoration." *Journal of Applied Ecology* 48: 600–608. https://doi.org/10.1111/j.1365-2664.2010.01925.x.

de Faria, S. M., G. P. Lewis, and J. I. Sprent. 1989. "Occurrence of Nodulation in the Leguminosae." *New Phytologist* 111: 607–619. https://doi.org/10.1111/j.1469-8137.1989.tb02354.x.

Denison, R. F., and E. T. Kiers. 2004. "Lifestyle Alternatives for Rhizobia: Mutualism, Parasitism, and Foregoing Symbiosis." *FEMS Microbiology Letters* 237: 187–193. https://doi.org/10.1016/j.femsle.2004.07.013.

Environment Canada. 2022. "Daily Data Report: London A. National Climate Data and Information Archive."

Farrell, A. D., J. C. Clifton-Brown, I. Lewandowski, and M. B. Jones. 2006. "Genotypic Variation in Cold Tolerance Influences the Yield of *Miscanthus*." *Annals of Applied Biology* 149: 337–345. https://doi.org/10.1111/j.1744-7348.2006.00099.x.

Gao, D., X. Wang, S. Fu, and J. Zhao. 2017. "Legume Plants Enhance the Resistance of Soil to Ecosystem Disturbance." *Frontiers in Plant Science* 8: 1295. https://doi.org/10.3389/fpls.2017.01295.

Gaul, D., D. Hertel, and C. Leuschner. 2008. "Effects of Experimental Soil Frost on the Fine-Root System of Mature Norway Spruce." *Journal of Plant Nutrition and Soil Science* 171: 690–98. https://doi.org/10.1002/jpln.200700284.

González, E. M., E. Larrainzar, D. Marino, S. Wienkoop, E. Gil-Quintana, and C. Arrese-Igor. 2015. "Physiological Responses of N_2-Fixing Legumes to Water Limitation." In *Legume Nitrogen Fixation in a Changing Environment*, edited by S. Sulieman and L. S. Tran. Dordrecht: Springer, Cham. https://doi.org/10.1007/978-3-319-06212-9_2.

Groffman, P. M., C. T. Driscoll, T. J. Fahey, J. P. Hardy, R. D. Fitzhugh, and G. L. Tierney. 2001. "Colder Soils in a Warmer World: A Snow Manipulation Study in a Northern Hardwood Forest Ecosystem." *Biogeochemistry* 56: 135–150. https://doi.org/10.1023/A:1013039830323.

Hagerty, T. P., and M. S. Kingston. 1992. "The Soils of Middlesex County." Ontario Ministry of Agriculture and Food. Map 3.

Henry, H. A. L. 2008. "Climate Change and Soil Freezing: Historical Trends and Projected Changes." *Climatic Change* 87: 421–434. https://doi.org/10.1007/s10584-007-9322-8.

Henry, H. A. L., M. Abedi, C. L. Alados, K. H. Beard, L. H. Fraser, A. Jentsch, J. Kreyling, et al. 2018. "Increased Soil Frost Versus Summer Drought as Drivers of Plant Biomass Responses to Reduced Precipitation: Results from a Globally Coordinated Field Experiment." *Ecosystems* 21: 1432–44.

Irshad, A., R. N. U. Rehman, H. A. Kareem, P. Yang, and T. Hu. 2021. "Addressing the Challenge of Cold Stress Resilience with the Synergistic Effect of *Rhizobium* Inoculation and Exogenous Melatonin Application in *Medicago truncatula*." *Ecotoxicology and Environmental Safety* 226: 112816.

Iwata, S., T. Tabuchi, and B. P. Warkentin. 1995. *Soil-Water Interactions: Mechanisms and Applications*, 2nd ed. New York, NY: Marcel Dekker, Inc.

JMP®, Version 17. 1989–2023. *Statstical Software*. Cary, NC: SAS Institute Inc.

Jonasson, S. 1986. "Influence of Frost Heaving on Soil Chemistry and on the Distribution of Plant Growth Forms." *Geografiska Annaler: Series A, Physical Geography* 68: 185–195. https://doi.org/10.1080/04353676.1986.11880172.

Joseph, G., and H. A. L. Henry. 2008. "Soil Nitrogen Leaching Losses in Response to Freeze-Thaw Cycles and Pulsed Warming in a Temperate Old Field." *Soil Biology and Biochemistry* 40: 1947–53. https://doi.org/10.1016/j.soilbio.2008.04.007.

Kiers, E. T., and R. F. Denison. 2008. "Sanctions, Cooperation, and the Stability of Plant-Rhizosphere Mutualisms." *Annual Review of Ecology, Evolution, and Systematics* 39: 215–236. https://doi.org/10.1146/annurev.ecolsys.39.110707.173423.

Komac, B., C. Pladevall, J. Peñuelas, J. V. Conesa, and M. Domènech. 2015. "Variations in Functional Diversity in Snowbed Plant Communities Determining Snowbed Continuity." *Plant Ecology* 216: 1257–74. https://doi.org/10.1007/s11258-015-0506-4.

Kreyling, J. 2010. "Winter Climate Change: A Critical Factor for Temperate Vegetation Performance." *Ecology* 91: 1939–48. https://doi.org/10.1890/09-1160.1.

Kreyling, J., M. Haei, and H. Laudon. 2012. "Absence of Snow Cover Reduces Understory Plant Cover and Alters Plant Community Composition in Boreal Forests." *Oecologia* 168: 577–587. https://doi.org/10.1007/s00442-011-2092-z.

Lebauer, D. S., and K. K. Treseder. 2008. "Nitrogen Limitation of Net Primary Productivity in Terrestrial Ecosystems Is Globally Distributed." *Ecology* 89: 371–79. https://doi.org/10.1890/06-2057.1.

Li, R., J. J. Volenec, B. C. Joern, and S. M. Cunningham. 1996. "Seasonal Changes in Nonstructural Carbohydrates, Protein, and Macronutrients in Roots of Alfalfa, Red Clover, Sweetclover, and Birdsfoot Trefoil." *Crop Science* 36: 617–623. https://doi.org/10.2135/cropsci1996.0011183X003600030016x.

Liu, Y. S., J. C. Geng, X. Y. Sha, Y. X. Zhao, T. M. Hu, and P. Z. Yang. 2019. "Effect of *Rhizobium* Symbiosis on Low-Temperature Tolerance and Antioxidant Response in Alfalfa (*Medicago sativa* L.)." *Frontiers in Plant Science* 10: 538. https://doi.org/10.3389/fpls.2019.00538.

Lorin, M., M.-H. Jeuffroy, A. Butier, and M. Valantin-Morison. 2016. "Undersowing Winter Oilseed Rape with Frost-Sensitive Legume Living Mulch: Consequences for Cash Crop Nitrogen Nutrition." *Field Crops Research* 193: 24–33. https://doi.org/10.1016/j.fcr.2016.03.002.

Lubbe, F. C., and H. A. L. Henry. 2020. "The Role of Perennation Traits in Plant Community Soil Frost Stress Responses." *Annals of Botany* 126: 873–881. https://doi.org/10.1093/aob/mcaa104.

Lubbe, F. C., J. Klimešová, and H. A. L. Henry. 2021. "Winter Belowground: Changing Winters and the Perennating Organs of Herbaceous Plants." *Functional Ecology* 35: 1627–39. https://doi.org/10.1111/1365-2435.13858.

Malyshev, A. V., and H. A. L. Henry. 2012a. "N Uptake and Growth Responses to Sub-Lethal Freezing in the Grass *Poa pratensis* L." *Plant and Soil* 360: 175–185. https://doi.org/10.1007/s11104-012-1233-4.

Malyshev, A. V., and H. A. L. Henry. 2012b. "Frost Damage and Winter Nitrogen Uptake by the Grass *Poa pratensis* L.: Consequences for Vegetative Versus Reproductive Growth." *Plant Ecology* 213: 1739–47. https://doi.org/10.1007/s11258-012-0127-0.

Marino, D., P. Frendo, R. Ladrera, A. Zabalza, A. Puppo, C. Arrese-Igor, and E. M. González. 2007. "Nitrogen Fixation Control under Drought Stress. Localized or Systemic?" *Plant Physiology* 143: 1968–74. https://doi.org/10.1104/pp.106.097139.

Martínez-Romero, E., and J. Caballero-Mellado. 1996. "*Rhizobium* Phylogenies and Bacterial Genetic Diversity." *Critical Reviews in Plant Sciences* 15: 113–140. https://doi.org/10.1080/07352689.1996.10393183.

Matamoros, M. A., L. M. Baird, P. R. Escuredo, D. A. Dalton, F. R. Minchin, I. Iturbe-Ormaetxe, M. C. Rubio, J. F. Moran, A. J. Gordon, and M. Becana. 1999. "Stress-Induced Legume Root Nodule Senescence. Physiological, Biochemical, and Structural Alterations." *Plant Physiology* 121: 97–112. https://doi.org/10.1104/pp.121.1.97.

McKersie, B. D., S. R. Bowley, and K. S. Jones. 1999. "Winter Survival of Transgenic Alfalfa Overexpressing Superoxide Dismutase." *Plant Physiology* 119: 839–848.

McKersie, B. D., Y. Chen, M. de Beus, S. R. Bowley, C. Bowler, D. Inze, K. D'Halluin, and J. Botterman. 1993. "Superoxide Dismutase Enhances Tolerance of Freezing Stress in Transgenic Alfalfa (*Medicago sativa* L.)." *Plant Physiology* 103: 1155–63.

Mekis, E., and L. A. Vincent. 2011. "An Overview of the Second Generation Adjusted Daily Precipitation Dataset for Trend Analysis in Canada." *Atmosphere-Ocean* 49: 163–177. https://doi.org/10.1080/07055900.2011.583910.

Meyer, D. W., and M. Badaruddin. 2001. "Frost Tolerance of Ten Seedling Legume Species at Four Growth Stages." *Crop Science* 41: 1838–42. https://doi.org/10.2135/cropsci2001.1838.

Middleton, E. L., and J. D. Bever. 2012. "Inoculation with a Native Soil Community Advances Succession in a Grassland Restoration." *Restoration Ecology* 20: 218–226. https://doi.org/10.1111/j.1526-100X.2010.00752.x.

Mir, A. H., M. A. Bhat, S. A. Dar, P. A. Sofi, N. A. Bhat, and R. R. Mir. 2021. "Assessment of Cold Tolerance in Chickpea (*Cicer* spp.) Grown under Cold/Freezing Weather Conditions of North-Western Himalayas of Jammu and Kashmir, India." *Physiology and Molecular Biology of Plants* 27: 1105–18.

Neuner, G. 2014. "Frost Resistance in Alpine Woody Plants." *Frontiers in Plant Science* 5: 654. https://doi.org/10.3389/fpls.2014.00654.

Orsenigo, S., A. Mondoni, G. Rossi, and T. Abeli. 2014. "Some like it Hot and Some Like It Cold, but Not Too Much: Plant Responses to Climate Extremes." *Plant Ecology* 215: 677–688. https://doi.org/10.1007/s11258-014-0363-6.

Palmborg, C., M. Scherer-Lorenzen, A. Jumpponen, G. Carlsson, K. Huss-Danell, and P. Högberg. 2005. "Inorganic Soil Nitrogen under Grassland Plant Communities of Different Species Composition and Diversity." *Oikos* 110: 271–282. https://doi.org/10.1111/j.0030-1299.2005.13673.x.

Perfect, E., R. D. Miller, and B. Burton. 1987. "Root Morphology and Vigor Effects on Winter Heaving of Established Alfalfa." *Agronomy Journal* 79: 1061–67. https://doi.org/10.2134/agronj1987.00021962007900060023x.

Perfect, E., R. D. Miller, and B. Burton. 1988. "Frost Upheaval of Overwintering Plants: A Quantitative Field Study of the

Displacement Process." *Arctic and Alpine Research* 20: 70–75. https://doi.org/10.1080/00040851.1988.12002652.

Prévost, D., P. Drouin, and H. Antoun. 1999. "The Potential Use of Cold-Adapted Rhizobia to Improve Symbiotic Nitrogen Fixation in Legumes Cultivated in Temperate Regions." In *Biotechnological Applications of Cold-Adapted Organisms*, edited by R. Margesin and F. Schinner, 161–176. Berlin/Heidelberg: Springer. https://doi.org/10.1007/978-3-642-58607-1_11.

Prévost, D., P. Drouin, S. Laberge, A. Bertrand, J. Cloutier, and G. Lévesque. 2003. "Cold-Adapted Rhizobia for Nitrogen Fixation in Temperate Regions." *Canadian Journal of Botany* 81: 1153–61. https://doi.org/10.1139/b03-113.

Rapacz, M., Å. Ergon, M. Höglind, M. Jørgensen, B. Jurczyk, L. Østrem, O. A. Rognli, and A. M. Tronsomo. 2014. "Overwintering of Herbaceous Plants in a Changing Climate. Still More Questions than Answers." *Plant Science* 225: 34–44. https://doi.org/10.1016/j.plantsci.2014.05.009.

Ritchie, M. E., and D. Tilman. 1995. "Responses of Legumes to Herbivores and Nutrients during Succession on a Nitrogen-Poor Soil." *Ecology* 76: 2648–55. https://doi.org/10.2307/2265835.

Rycroft, S., and H. Henry. 2025. "Data from: High Sensitivity of Herbaceous Legumes to Freezing: Insights from a Multi-Year Snow Removal Study." Dataset. Dryad. https://doi.org/10.5061/dryad.xwdbrv1nw.

Rycroft, S. L. 2023. "Freezing Tolerance of Herbaceous Legumes Within Southwestern Ontario: Evidence of Disproportionate Freezing Sensitivity." PhD Thesis. University of Western Ontario, London, Ontario, Canada.

Rycroft, S. L., and H. A. L. Henry. 2024. "High Freezing Sensitivity of Legumes Relative to Other Herbaceous Species in Northern Temperate Plant Communities." *Annals of Botany* 134: 283–294. https://doi.org/10.1093/aob/mcae072.

Sherwood, J. A., D. M. Debinski, P. C. Caragea, and M. J. Germino. 2017. "Effects of Experimentally Reduced Snowpack and Passive Warming on Montane Meadow Plant Phenology and Floral Resources." *Ecosphere* 8: e01745. https://doi.org/10.1002/ecs2.1745.

Smith, E. T., and S. C. Sheridan. 2018. "The Characteristics of Extreme Cold Events and Cold Air Outbreaks in the Eastern United States." *International Journal of Climatology* 38: e807–e820. https://doi.org/10.1002/joc.5408.

Sprent, J. I., J. Ardley, and E. K. James. 2017. "Biogeography of Nodulated Legumes and Their Nitrogen-Fixing Symbionts." *New Phytologist* 215: 40–56. https://doi.org/10.1111/nph.14474.

Steponkus, P. L., M. Uemura, and M. S. Webb. 1993. "A Contrast of the Cryostability of the Plasma Membrane of Winter Rye and Spring Oat—Two Species that Widely Differ in their Freezing Tolerance and Plasma Membrane Lipid Composition." In *Advances in Low-Temperature Biology*, Vol. 2, edited by P. L. Steponkus, 211–312. London, UK: JAI Press, Ltd.

Stover, H. J., and H. A. L. Henry. 2019. "Interactions Between Soil Heterogeneity and Freezing: Implications for Grassland Plant Diversity and Relative Species Abundances." *Global Change Biology* 25: 2275–84. https://doi.org/10.1111/gcb.14645.

Stover, H. J., and H. A. L. Henry. 2020. "Soil Homogenization Modifies Productivity, Nitrogen Retention and Decomposition in Restored Grassland." *Ecosystems* 23: 264–277. https://doi.org/10.1007/s10021-019-00401-w.

Thurston, C. L., J. M. Grossman, R. Fudge, J. E. Maul, S. Mirsky, and N. Wiering. 2022. "Cold Stress Reduces Nodulation and Symbiotic Nitrogen Fixation in Winter Annual Legume Cover Crops." *Plant and Soil* 481: 661–676. https://doi.org/10.1007/s11104-022-05667-z.

Tierney, G. L., T. J. Fahey, P. M. Groffman, J. P. Hardy, R. D. Fitzhugh, and C. T. Driscoll. 2001. "Soil Freezing Alters Fine Root Dynamics in a Northern Hardwood Forest." *Biogeochemistry* 56: 175–190. https://doi.org/10.1023/A:1013072519889.

Turkington, R. A., and G. D. Franko. 1980. "The Biology of Canadian Weeds. 41. *Lotus corniculatus* L." *Canadian Journal of Plant Science* 60: 965–979. https://doi.org/10.4141/cjps80-140.

Vankoughnett, M. R., and H. A. L. Henry. 2014. "Soil Freezing and N Deposition: Transient vs Multi-Year Effects on Plant Productivity and Relative Species Abundance." *New Phytologist* 202: 1277–85. https://doi.org/10.1111/nph.12734.

Vankoughnett, M. R., D. A. Way, and H. A. L. Henry. 2016. "Late Winter Light Exposure Increases Summer Growth in the Grass *Poa pratensis*: Implications for Snow Removal Experiments and Winter Melt Events." *Environmental and Experimental Botany* 131: 32–38. https://doi.org/10.1016/j.envexpbot.2016.06.014.

Vincent, L. A., X. Zhang, É. Mekis, H. Wan, and E. J. Bush. 2018. "Changes in Canada's Climate: Trends in Indices Based on Daily Temperature and Precipitation Data." *Atmosphere-Ocean* 56: 332–349. https://doi.org/10.1080/07055900.2018.1514579.

Vitousek, P. M., and R. W. Howarth. 1991. "Nitrogen Limitation on Land and in the Sea: How Can it Occur?" *Biogeochemistry* 13: 87–115. https://doi.org/10.1007/BF00002772.

Vyse, K., M. Pagter, E. Zuther, and D. K. Hincha. 2019. "Deacclimation after Cold Acclimation-a Crucial, but Widely Neglected Part of Plant Winter Survival." *Journal of Experimental Botany* 70: 4595–4604. https://doi.org/10.1093/jxb/erz229.

Wisniewski, M., I. R. Willick, and L. V. Gusta. 2017. "Freeze Tolerance and Avoidance in Plants." In *Plant Stress Physiology*, edited by S. Shabala, 279–299. Oxfordshire, UK: CABI.

Yadav, S. K. 2010. "Cold Stress Tolerance Mechanisms in Plants. A Review." *Agronomy for Sustainable Development* 30: 515–527. https://doi.org/10.1051/agro/2009050.

Zahran, H. H. 1999. "*Rhizobium*-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. 1999." *Microbiology and Molecular Biology Reviews* 63: 968–989. https://doi.org/10.1128/MMBR.63.4.968-989.1999.

Zhao, J., X. Wang, X. Wang, and S. Fu. 2014. "Legume-Soil Interactions: Legume Addition Enhances the Complexity of the Soil Food Web." *Plant and Soil* 385: 273–286. https://doi.org/10.1007/s11104-014-2234-2.

Zhao, J., Z. Zeng, X. He, H. Chen, and K. Wang. 2015. "Effects of Monoculture and Mixed Culture of Grass and Legume Forage Species on Soil Microbial Community Structure under Different Levels of Nitrogen Fertilization." *European Journal of Soil Biology* 68: 61–68. https://doi.org/10.1016/j.ejsobi.2015.03.008.

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