Mycorrhizal inoculation mitigates damage from an intermediate, but not severe, frost event for a cool-season perennial bunchgrass

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Abstract: Extreme cold events can damage plant tissues, altering growth and reproduction. Soil fungi may help plants tolerate environmental stressors, but the role these microbes play during episodes of severe cold warrants further examination. Using the bunchgrass *Elymus canadensis* L., we tested how inoculation with mycorrhizal fungi alters plant tolerance to freezing temperatures (tested at –8 °C and –16 °C). We found that, regardless of mycorrhizal inoculation, *E. canadensis* exposed to –16 °C exhibited greater tissue damage, less tiller growth, and fewer reproductive tillers than plants exposed to the control or –8 °C conditions. Plants exposed to –8 °C and –16 °C displayed greater levels of visible damage compared with the control plants. Mycorrhizae reduced damage to tillers in the –8 °C treatment, but had less effect on tiller damage in the control or –16 °C treatments. Inoculation with arbuscular mycorrhizal fungi limited the tiller number for *E. canadensis*, but only at the control temperature, suggesting that mycorrhizae may impose costs on *E. canadensis* under benign thermal conditions. Our study demonstrates that extreme temperatures can affect multiple components of growth in *E. canadensis*, and that the costs and benefits of arbuscular mycorrhizal fungi, where found, depend upon the thermal environment. Our findings reinforce the overarching importance of historically rare, but increasingly common, environmental extremes in shaping the growth of plants.

Key words: cold damage, false spring events, frost tolerance, microbial mutualists, winter climate.

Résumé : Les épisodes de froid extrême peuvent endommager les tissus végétaux, affectant la croissance et la reproduction. Les champignons du sol peuvent aider les plantes à tolérer des agresseurs environnementaux, mais le rôle que ces microbes exercent durant des épisodes de grand froid doit être davantage étudié. En utilisant la graminée cespitueuse, *Elymus canadensis* L. (élyme du Canada), les auteurs ont testé comment l’inoculation de champignons mycorhiziens modifie la tolérance du plant à des températures sous le point de congélation (–8 °C et –16 °C). Ils ont trouvé que peu importe l’inoculation de mycorhize, *E. canadensis* exposé à –16 °C présentait un dommage tissulaire plus important, une croissance des talles moins élevée et une diminution du nombre de talles reproductrices comparativement aux plants soumis aux conditions contrôles ou à –8 °C. Les plants exposés à –8 °C ou –16 °C montraient des niveaux plus élevés de dommages visibles comparativement aux plants contrôles. Les mycorhizes réduisaient les dommages causés aux talles par le traitement à –8 °C, mais elles avaient moins d’effet sur le dommage aux talles à la suite du traitement contrôle ou à –16 °C. L’inoculation de champignons mycorhiziens à arbuscules (CMA) limitait le nombre de talles d’*E. canadensis*, mais seulement à la température contrôle, suggérant que les mycorhizes peuvent imposer certains coûts à *E. canadensis* soumis à des conditions thermiques plus douces. Cette étude démontre que les températures extrêmes peuvent affecter différentes composantes de la croissance de *E. canadensis*, et que les coûts et bénéfices des CMA, lorsqu’observés, dépendent de l’environnement thermique. Ces données renforcent l’importance primordiale des conditions environnementales extrêmes, historiquement rares, mais de plus en plus fréquentes, dans le façonnement de la croissance des végétaux. [Traduit par la Rédaction]

Mots-clés : dommage provoqué par le froid, fausse arrivée du printemps, tolérance au gel, mutualistes microbiens, climat hivernal.
Introduction

Spring frost events can structure plant populations by reducing plant growth or reproduction (Gu et al. 2008; Augspurger 2009; Hufkens et al. 2012). Changes in global climate will likely advance spring phenology (Lebourgeois et al. 2010; Fu et al. 2012; Ladwig et al. 2019), further increasing the likelihood of spring frost damage during “false spring” events (Inouye 2008; Kreyling 2010; Augspurger 2013; Penczykowski et al. 2017; Kral-O’Brien et al. 2019). Increasing likelihood of spring frost damage can generate ecological and economic consequences for natural plant communities and agricultural plant productivity (e.g., Gu et al. 2008; Augspurger 2009). Many factors influence plant susceptibility to cold (e.g., plant ontogeny, Vitas et al. 2014; exposure to drought, Kreyling et al. 2012), and recent work suggests that cues connected with biotic interactions may also play an important role influencing a plant’s response to cold (e.g., plant hormones associated with herbivory, Connolly and Orrock 2018). Given the potential importance of biotic interactions in shaping plant responses to cold stress, examining how cold stress and species interactions might interact to modify plant performance may provide important insight into how plants respond to novel thermal regimes. Without accounting for interactions between abiotic and biotic forces, we may over- or underestimate species’ responses to climate change (Urban et al. 2016).

Infection by arbuscular mycorrhizal (AM) fungi can play an important role in the growth, development, reproduction, and stress tolerance of their host plants (Smith and Read 2002; Parniske 2008; van der Heijden et al. 2010). AM fungi can facilitate plant resistance to environmental stresses such as herbivory by mammals (Gange and West 1994; Connolly et al. 2016), attack by soil pathogens (Smith and Read 2002), low soil water potential (Augé 2001), and haline edaphic conditions (Evelin et al. 2009). Inoculation with AM fungi may also provide physiological tolerance to extreme cold in perennial grasses (i.e., modifying photosynthetic capacity, Zhu et al. 2010; reduce the extent of oxidative damage; Chu et al. 2016). Alternatively, certain environmental conditions (e.g., low soil phosphorus) may require arbuscular mycorrhizae to use a large fraction of fixed carbon dioxide to sustain nutrient uptake for the host plant (Smith and Read 2002). The partitioning of these metabolites to AM fungi may limit the carbon available to plants to recover tissue and resume growth following exposure to cold extremes. Currently, it is unclear how soil inoculation by AM fungi will influence the capacity of plants to maintain growth and reproductive output following severe freeze-events.

In this study, we use an experimental approach that pairs simulated spring freeze events at two severities (–8 °C and –16 °C) with an AM fungi soil inoculation treatment to evaluate how putative soil mutualists may shape plant responses to extreme events. We focused on the potential interactive effects of soil inoculation and frost severity on the growth and reproductive response of the bunchgrass, *Elymus canadensis*. *Elymus canadensis* is a common cool-season perennial species widely distributed across the temperate North America, including the northern temperate regions likely to undergo significant increases in frost occurrence and severity (Kreyling 2010). We specifically seek to evaluate how a cool-season grass — characterized in part by rapid growth during spring — is affected by climate change phenomena occurring during those periods of growth (i.e., spring freeze events). Perennial grasses, including *Elymus* spp., can form mycorrhizal mutualistic relationships with AM fungi (Wilson and Hartnett 1998; Anderson 2008), and previous work suggests that *E. canadensis* is likely a reliable indicator of the extent to which freezing temperatures can influence plant performance and plant–microbe interactions within the soil (Connolly and Orrock 2015). By examining the effects of AM fungi on the response of *E. canadensis* to freezing temperatures, we expected to gain insight into how the increased likelihood of frost events occurring after spring dehardening may influence plant performance and reproductive capacity.

Materials and methods

*Elymus canadensis* seeds were purchased from Agrecol Native Nursery (Evansville, Wisconsin, USA). The seeds were grown in Waushara County (Wisc.), indicating that the source *E. canadensis* populations were adapted to current northern temperate winter climate conditions. On 2 February 2016, three *E. canadensis* seeds were sown (~0.5 cm deep) in circular pots (6.5 cm diameter × 24.5 cm deep) filled with potting soil mix (Metromix 360; SunGro Horticulture, Agawam, Massachusetts, USA). The potting soil medium was homogenized prior to filling the circular pots to standardize the soil microbial communities residing across all treatments; the Metromix 360 soil medium is a mix of sphagnum peat moss, bark, vermiculite, dolomitic limestone, and a wetting agent. We did not autoclave the mix before potting because autoclaving may generate artificial flushes of minerals that could artificially modify seedling growth patterns (Endlweber and Schue 2006) or possibly alter the soil nutrient balance in such a way as to minimize the colonization of *E. canadensis* seedlings by AM fungi. The colonization of roots by mycorrhizae in unsterilized Metro Mix can occur, but the colonization rates are low (Gorman and Starrett 2003).

The soil in 48 pots was inoculated with a commercial mycorrhizal inoculum (Mike O’Rizey Granular; Plant Revolution Inc., Santa Ana, California, USA) prior to seed sowing. We deposited approximately 0.667 ± 0.014 g of the mycorrhizal mix in each of three 3 cm deep holes in each pot (2.0 g per pot) and then backfilled each hole. We made similar holes in the soil of another 48 control pots, but no mycorrhizal inoculum was added, and all of the
holes were backfilled. Each gram (1 g) of granular inoculum contained 33 spores of each of four different AM fungal species: *Rhizophagus intraradices*, *Funneliformis mosseae*, *Rhizophagus aggregatus*, and *Claroideoglomus etunicatum* (264 spores/pot; naming conventions follow Schüßler and Walker 2010; Walker 2016). AM fungi are typically generalist in host selection (Smith and Read 2002), and research suggests that *Elymus canadensis* forms associations with *Funneliformis mosseae* (Noyd et al. 1995) and other mycorrhizal species (Hetrick et al. 1994; Wilson and Hartnett 1998; Anderson 2008), indicating that our chosen inoculum introduces an appropriate suite of AM fungal species for interaction with the experimental plant species. We watered the pots daily and allowed water to drain freely, to mimic natural water infiltration and prevent cross-contamination. On 15 February 2016, the seedlings were thinned to the single largest individual per pot. The seedlings were grown in a greenhouse maintained at 25 °C and supplemental lighting was provided to maintain a 12–12 h light–dark photoperiod.

On 1 April 2016, the plants were transferred to a greenhouse with a 20/15 °C temperature cycle and a 12–12 light–dark photoperiod (light intensity 50–60 μmol quanta·m⁻²·s⁻¹). On 25 April 2016, the plants were divided into three groups and placed in one of three environmental growth chambers at the University of Wisconsin (Madison, Wisc.) Biotron facility [32 plants per room; average maximum tiller length at this time: 12.16 ± 0.16 cm (mean ± SE); average tiller number at this time point = 3.73 ± 0.14 tillers]. We did not assess the status of mycorrhizae on *E. canadensis* prior to freeze treatment application. However, in a similar study, Chu et al. (2016) reported >30% root colonization by *Funneliformis mosseae* of the congener *Elymus nutans* 60 days after inoculation with AM fungal spores. Our plants were at 83 days after the inoculation treatment when we transferred them to the growth chambers, suggesting that sufficient time had elapsed for root colonization to occur prior to the temperature treatments.

To facilitate natural freezing (Henry 2007), the pots were embedded in large plastic tubs filled with medium-coarse grade vermiculite so that the plant soil surface in each pot was level with the vermiculite. As photoperiod and temperature play an important role regulating cold tolerance in both plants and mycorrhizal fungi (Gray et al. 1997; Addy et al. 1998), each room ran an identical nine-week program of temperature and photoperiod regimes to simulate climate transitions from fall to winter, and from winter to spring. The plants were introduced to the chambers and maintained for 7 days at a 20 °C/15 °C temperature regime with a 12–12 h light–dark photoperiod, mimicking the conditions experienced in their most recently occupied greenhouse. On 2 May 2016, chambers cooled to a 10 °C/3 °C temperature regime with a 10–14 h light–dark photoperiod; these conditions were maintained for three weeks. On 21 May 2016, the environmental chamber conditions were cooled again to a 3 °C/1 °C temperature regime with a 10–14 h light–dark photoperiod; these conditions were maintained for four weeks. On 20 June 2016, the environmental growth chamber conditions were warmed to a 12 °C/8 °C “false spring” temperature regime with a 14–10 h light–dark photoperiod for seven days. Temperature and photoperiod set points reflect approximate fall and subnivean conditions measured at locations throughout temperate Wisconsin (B.M. Connolly, unpublished data); see Supplemental Information S1 for a table summarizing the environmental chamber treatment structure (Supplementary data, Table S1).

From 27–28 June 2016, each room ran one of three experimental frost treatments using set points to approximate: (i) no frost event (constant 7 °C); (ii) moderate frost event (temperature declined to ~8 °C at a rate of ~4 °C per hour); and (iii) severe frost event (temperature declined to ~16 °C at a rate of ~5 °C per hour). Minimum temperatures were maintained for approximately 6 h and then warmed back to 8 °C at a rate of 4–5 °C per hour. Following the frost event, the plants were returned to a 15/12 °C temperature regime and a 14–10 h photoperiod from 29 June 2016 to 1 July 2016. On 1 July 2016, the plants were returned to a greenhouse maintained at a constant 25 °C and received supplemental lighting to maintain a 12–12 h light–dark photoperiod. Minimum temperature set points were selected to reflect long-term patterns of minimum air temperature recorded at two agricultural research stations located in Wisconsin: Arlington Research Station, and Hancock Research Station (Guiden et al. 2018); see Supplemental Information S1 for the freezing treatment temperature data (Fig. S1). On 2 June 2016, an equipment malfunction in one chamber resulted in a brief departure from set parameters. The plants were re-randomized to different rooms following the growth chamber malfunction, and subsequent data analysis suggested that there were no significant effects from this brief temperature anomaly on our findings (Supplemental Information S2; see also Guiden et al. 2018).

Data collection

On 29 June 2016, approximately 36 h after the conclusion of the simulated frost event, 30 plants (five replicates of each mycorrhizal inoculation × frost severity treatment combination) were collected to estimate plant tissue cold damage using proportion electrolyte leakage (PEL). To estimate PEL, the longest tiller of each individual was cut 15 cm above the soil surface. Four segments

of the leaf tissue (1 cm long) were rinsed with Millipore-filtered water and were placed in a microfuge tube with 1 mL of Millipore-filtered water. The tubes were agitated for 20 min in a tabletop shaker. The solute concentration of each sample was then measured with a 4 mm conductivity probe (ELpostfreeze, InLab 751; Mettler–Toledo International Inc., Greifensee, Switzerland). The samples were then placed in boiling water for 20 min, allowed to cool to room temperature, and sampled again with the conductivity probe to estimate the maximum damage to plant leaf tissue (ELpostboil). We used the quotient of ELpostfreeze to ELpostboil to estimate proportion electrolyte leakage (Connolly and Orrock 2018). On 13 July 2016, two weeks after the simulated frost, we counted the number of tillers that exhibited > 10% damage (e.g., leaf curling, wilting, severe necrosis), maximum tiller length, and the total number of tillers on each plant. Visual determinations can reliably estimate cold damage to plants (Neuner and Buchner 1999; Pescador et al. 2018); the same observer (B.M.C.) conducted all of the counts and damage estimates following a blind protocol. On 24 August 2016, approximately 8 weeks after the frost event, we measured the maximum length of the longest three tillers on each individual, and counted the total number of tillers and the number of tillers bearing reproductive glumes for each plant. For perennial grasses under natural conditions, the total number of tillers and the number of reproductive tillers are affected by treatment with commercial mycorrhizal inoculum and can correlate with greater perennial grass productivity (e.g., Connolly et al. 2016).

Data analysis

We used general linear models to evaluate how simulated frost treatments, mycorrhizal inoculum, and the interaction of these two fixed effects the proportion of leaf electrolyte leakage, composite visual tiller damage estimates, maximum tiller length at eight weeks post-freeze, and total tiller number at eight weeks post-freeze in influenced E. canadensis. The proportion of electrolyte leakage was logit-transformed (Warton and Hui 2011). We used the product of the proportion of visible tiller damage and maximum tiller length at the time of visual damage estimates to quantify foliar damage at two weeks after the freeze treatment; this composite metric allowed us to account for differences in plant size at the time of visual cold damage estimates. We applied a Box Cox transformation (λ ≈ 3) to maximum tiller length at eight weeks post-freeze to meet model normality assumptions (Crawley 2013). Finally, we used a generalized linear model with a binomial response distribution to evaluate how simulated frost treatments, addition of mycorrhizal inoculum, and the interaction of these two fixed effects influenced the proportion of reproductive tillers present on each plant at eight weeks after the frost treatment. Supplementary PERMANOVA analysis of plant growth responses reveals similar responses to treatments as those determined by linear models (See Supplemental Information S3). We used program R (R Core Team 2019) to conduct all data analyses (packages: “lme4”, Bates et al. 2015; “emmeans”, Lenth 2019; “car”, Fox and Weisberg 2011).

Results

The proportion of electrolyte leakage (PEL) differed significantly between the freeze treatment levels [Fig. 1A; Freeze treatment (FRZ): F1,24 = 23.62, p < 0.001]; PEL for plants assigned the –16 °C freeze treatment were two-fold greater than the PEL values from both the control temperature and the –8 °C freeze treatment (all p values < 0.001). PEL did not differ between plants from the control temperature and plants in the –8 °C freeze treatment (t = –1.10, df = 24, p = 0.526). The presence of mycorrhizal inoculum did not influence plant PEL [Mycorrhizae treatment (MYCO): F1,24 = 1.78, p = 0.194] and the effects of mycorrhizal inoculum did not differ between freeze treatments (FRZ × MYCO interaction: F1,24 = 0.698, p = 0.507).

Composite visual tiller damage estimates for E. canadensis were greater in the group subjected to –16 °C (Fig. 1B; FRZ: F2,60 = 14.41, p < 0.001). The control plants had significantly less visible damage than plants from the –8 °C freeze treatment group (control vs. –8 °C; t = –4.52, df = 60, p < 0.001) or the –16 °C freeze treatment group (control vs. –16 °C; t = –4.77, df = 60, p < 0.001). Cold damage did not differ between the –8 °C and –16 °C freeze treatment levels (t = –0.26, df = 60, p = 0.965). Plants inoculated with mycorrhizal fungi displayed less visible tiller damage [MYCO: F1,60 = 11.79, p = 0.001]. Soil inoculation with mycorrhizae corresponded to a >50% reduction in composite visual tiller damage estimates for the control temperature treatment and the –8 °C freeze treatment, and >14% reduction in composite visual tiller damage estimates for the –16 °C freeze treatment. There was no significant interaction between mycorrhizal inoculation and freeze treatment level (FRZ × MYCO interaction: F1,60 = 0.24, p = 0.605).

Maximum tiller length and the proportion of reproductive tillers differed between freeze treatment levels (Figs. 1C and 1D; FRZ: tiller length, F1,60 = 19.00, p < 0.001; proportion reproductive tillers, χ² = 15.29, df = 2, p < 0.001). Treatment with the mycorrhizal inoculum did not modify E. canadensis maximum tiller length or the proportion of reproductive tillers per plant (MYCO: all p-values > 0.471), nor were these response variables influenced by a significant interaction between mycorrhizal inoculation and freeze treatment level (FRZ × MYCO interaction: all p-values > 0.412). Tiller length and the proportion reproductive tillers per plant did not differ between E. canadensis plants from the control and the –8 °C freeze treatment groups (tiller length: t = 0.03, df = 60, p = 0.980; proportion reproductive tillers: z = 0.92, p = 0.628). Both the control and the plants subjected to
−8 °C freeze had tillers that were 40% longer, and double the proportion of reproductive tillers per plant compared with the *E. canadensis* subjected to the −16 °C freeze treatment (all pairwise contrasts *p* < 0.011).

The effect of mycorrhizal inoculum on the number of *E. canadensis* tillers differed based on freeze-treatment level (Fig. 1E; MYCO × FRZ: *F*[2,60] = 3.34, *p* = 0.042). Within the control temperature treatment group, *E. canadensis* treated with commercial mycorrhizal inoculum had approximately 18% fewer tillers than *E. canadensis* not treated with the commercial mycorrhizal inoculum, but treatment with mycorrhizae did not influence tiller number for plants in the −8 °C or 16 °C freeze treatment groups (all *p* values > 0.251).

**Discussion**

Extreme thermal stress events can limit plant survival and growth ([Jentsch et al. 2007; Kreyling et al. 2008; Galiano et al. 2010; Kreyling 2010; but see Lloret et al. 2012]), and these extreme thermal events are likely to increase in frequency and intensity in the future ([IPCC 2014]). AM fungi may buffer terrestrial plants from thermal stress (i.e., as mutualists) or exacerbate the deleterious effects of thermal stress by taxing carbon stores; the
ecological role of AM fungi regarding plant performance following exposure to cold stress is still uncertain. Our work suggests that inoculation with AM fungi may reduce foliar damage associated with freezing, but the magnitude of this positive effect depends on frost severity. Restoration or management efforts using perennial grass seeding or transplanting may need to account for how false spring events can alter cool season perennial grass seedling survival and growth, particularly in northern temperate ecosystems where false spring events can be devastating (Gu et al. 2008; Augspurger 2009, 2013). Inoculation with mycorrhizae — particularly at sites with ineffective or reduced AM fungal spore soil banks (e.g., sites exposed to frequent flooding, fire, or soil disturbance) — may help to minimize cold damage to plants in frost-prone sites.

Cold-mediated damage directly influences individual plant performance (Burke et al. 1976) and may indirectly alter plant biotic interactions (e.g., inter- and intraspecific competition, plant–mutualist, plant–pathogen) and plant demography (Inouye 2008; Kreyling 2010; Connolly and Orrock 2015). In our study, frost treatments had strong direct effects on the growth and reproduction of E. canadensis — severe frost temperatures generated significant cellular damage in leaves, and truncated individual plant productivity and reproductive output. This specific finding, while not novel (e.g., Inouye 2008; Kreyling et al. 2012), contributes to our growing understanding of how temperate plants are likely to respond to novel temperature extremes and provides insight into how the legacies of extreme cold may influence individual plant performance and fate during the subsequent growing season (e.g., Connolly et al. 2017). For example, our severe spring frost treatment (−16 °C) resulted in 28% shorter E. canadensis compared with the control plants (Fig. 1C), supporting recent work indicating that severe frosts and false spring events can directly reduce plant growth and productivity (Hufkens et al. 2012; Guiden et al. 2018). Cold-mediated reductions in plant height and growth may influence a variety biotic interactions and demographic processes: altering plant competition dynamics by generating differential resource capture (e.g., light competition, Hautier et al. 2009), reducing forage availability to herbivores (e.g., insects, Castagneryrol et al. 2013; ungulates, Louthan et al. 2014), or altering plant demography by altering seed dispersal kernels (Thomson et al. 2011; but see Tamme et al. 2014).

For example, a 20 cm reduction in the height of reproductive tillers of E. canadensis, resulting from severe cold damage, could result in a 30% reduction in dispersal distance (estimated from Thomson et al. 2011). Compounded across multiple generations, lower seed dispersal distance may generate demographic consequences (e.g., density-dependent mortality) for this perennial species.

The growth of E. canadensis did not differ among the mycorrhizal inoculation treatments (Fig. 1C). These results suggest that either (i) AM fungi did not ameliorate the effects of cold-stress on growth of E. canadensis, or (ii) our treatment did not promote colonization by AM fungi. North American perennial cool-season grasses (e.g., Agropyron spp., Elymus spp.) typically display a muted — but not necessarily negligible — growth response to infection by AM fungi compared with warm-season grasses (Wilson and Hartnett 1998). Enhanced growth is only one possible benefit associated with infection by mycorrhizae; AM fungi may also indirectly shape patterns of plant growth under different edaphic conditions (Augé 2001; Evelin et al. 2009) or by altering plant interactions with herbivores (Gange and West 1994; Kempel et al. 2010; Connolly et al. 2016) and soil root pathogens (Newsham et al. 1995).

Alternatively, our study plants may have formed few root associations with the fungi in our inoculum. In their experiment using indigenous Glomus spp. spores to inoculate E. canadensis, Wilson and Hartnett (1998) indicate that E. canadensis has relatively low rates of root colonization (~15%) compared with 13 other perennial cool-season plants (root colonization range: 10%–52%, mean: 26%), and that E. canadensis displayed no appreciable change in aboveground biomass corresponding to mycorrhizal colonization of their roots. Similarly, Anderson (2008) found comparable levels of root colonization by AM fungi (5%–25%) on E. canadensis on restored prairie sites, and presented results indicating that root colonization by AM fungi on E. canadensis was lower at older restoration sites. Although we did not assess root colonization in our study, our results indicate that the AM fungal inoculation treatment influenced E. canadensis performance (i.e., reduced tiller production; Fig. 1E) and response to cold (i.e., minimizing foliar leaf damage; Fig. 1B) in patterns consistent with other studies evaluating the effects of confirmed colonization by AM fungi on the performance of perennial grasses (e.g., Wallace 1981; Skálová and Vosátka 1998; Chu et al. 2016). Additionally, mycorrhizal infection is often associated with greater root biomass in perennial grasses (e.g., Wallace 1981); root biomass collected from a subset of our constant temperature plants was marginally greater in mycorrhizae-treated plants (5.19 ± 0.25 g, least squares mean ± SE) compared with plants that did not receive mycorrhizae (4.37 ± 0.25 g; see Supplemental Information S41). Considered collectively, our results suggest our study plants did form associations with the AM fungi present in the commercial inoculum and were likely an effective tool for evaluating the role of AM fungi infection on perennial grass response to simulated cold treatments.

Conclusions and future directions

Extreme cold events during early spring — such as those simulated in our experiment — may become more common in northern temperate ecosystems, and are likely to generate unique consequences for temperate
perennial plants (Monson et al. 2006; Bardgett et al. 2013; Král-O’Brien et al. 2019). Future research, however, could investigate the interaction of plants, cold, and AM fungi under various edaphic contexts to understand when these interactions are likely to be affected by changes in early spring phenology. Soil freezing also influences the dormancy and efficacy of AM fungal hyphae. Some AM fungal hyphae retain the potential to remain infective in frozen soils (Addy et al. 1997), and future work could explore how winter-active AM fungal hyphae influence spring freeze tolerance in new emerged seedlings across important resource gradients (e.g., soil phosphorus availability). Additionally, soil microbes (e.g., AM fungi, ectomycorrhizal fungi) associating with plant roots may also be differentially and adversely affected by these novel thermal regimes at shallow soil depths (Klironomos et al. 2001; Tibbett and Cairney 2007), but diverse AM fungal communities can exist even at deep soil depths (e.g., Oehl et al. 2005). These deeper AM fungal communities may play a prominent role in perennial plant performance, as microbial communities in shallow soil environments are limited by novel cold temperature regimes. Understanding the factors driving variation in the responses of plants and their mutualists to extreme cold events promises to improve our ability to predict changes in plant communities exposed to novel climates.

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