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Disturbance Type and Timing Affect Growth and Tolerance Strategies in Grassland Plant Leaves☆,☆☆

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ABSTRACT

As human activities alter winter climates and disturbance regimes in grassland and rangeland ecosystems, the temperatures that plants experience during spring are changing. Litter can help buffer overwintering herbaceous plants from temperature fluctuations, and management practices dictate whether litter is present during the winter. Here, we investigate how disturbance type (burning, mowing) and timing (spring, fall) affect leaf characteristics related to growth and stress tolerance and how these traits change over time for five common tallgrass prairie species including four forb *(Monarda fistulosa, Ratibida pinnata, Silphium integrifolium, Symphiotrichum laeve)* and one grass species *(Bromus inermis).* To do this, we established a field experiment in Wisconsin, where plots were annually burned in the fall, mowed in the fall, burned in the spring, or left undisturbed (control) for 3 yr. We sampled leaves of target species seven times from spring emergence through early summer to measure specific leaf area (SLA) and leaf cold tolerance in each treatment. Leaves from fall-burned plots had lower SLAs, while leaves in spring-burned plots had higher SLAs early in the growing season. Leaf cold tolerance was similar across most treatments except in spring-burn plots, where leaves became more cold-hardy through time. We found weak evidence of a tradeoff between leaf growth and both cold tolerance and SLA. These results suggest that management decisions like litter removal before winter (e.g., fall burn or mow) prompted different plant responses compared with plots where litter was present during winter (e.g., spring burn). As species respond to winter climate change, management decisions have implications for mitigating climate change impacts and maintaining diversity in grasslands by affecting early-season plant growth strategies. For example, removing litter in the fall by burning promotes stress-tolerant responses, which may better equip plants to tolerate changing spring conditions.

Introduction

In temperate and polar regions, climate conditions are changing rapidly (IPCC 2014). This is especially true during winter, resulting in earlier snow melt, more variable winter soil temperatures (Groffman et al. 2001; Kreyling 2010; Williams et al. 2015), and greater potential for “false springs” where warm temperatures trigger plant growth but are followed by freezing temperatures that impact plant fitness (Augspurger 2013; Zohner et al. 2020). These changes to seasonal timing and soil temperatures have the potential to misalign plant responses to cold temperatures, resulting in extensive damage to plants (Charrier et al. 2015) and altered community composition (Inouye 2008; Kreyling 2010). The response of particular species and communities varies, however, depending on evolutionarily selected life history characteristics and the current rate of climate change (Ackerly et al. 2010).

In seasonal systems, the beginning of the growing season is a critical time for plant growth and investment tradeoffs (Vitasse et al. 2014). In temperate and polar climates, tolerance of cold temperatures is a constraint on plant fitness, biogeography, and community composition (Inouye 2000; Šímová et al. 2017; Wen et al. 2018; Zanne et al. 2018). To survive in cold places, plants must avoid or tolerate subzero temperatures while maintaining positive

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carbon gains. This can be done by emerging and activating growth earlier than neighboring plants, which can maximize resource capture and ultimately enhance growth and fecundity. However, early emergence can expose individuals to a greater risk of freezing, which can damage or kill sensitive tissues (Gu et al. 2008). This tradeoff between growing early and fast to fill space versus avoiding damage from freezing has strong impacts on plant survival, resulting in a variety of strategies for how plants respond to environmental cues in the spring (Vitasse et al. 2014).

Plants can alter their responses to spring conditions by changing when leaves emerge, how cold tolerant these early emerging leaves are, how fast they grow, and nutrient content in new leaves. The investments that plants make in young leaves are of critical importance as cold temperatures in spring can cause immediate damage (Inouye 2008) and longer-term impacts on growth, survival, and fecundity (Malyshev and Henry 2012; Vitasse et al. 2014; Guiden et al. 2018). While greater cold tolerance prevents damage, it can come at a cost to growth rate (Koehler et al. 2012) because it requires producing sugars and enzymes that help reduce ice formation (Zuther et al. 2015), protect membranes from damage, and reduce osmotic stress when extracellular ice forms (Rapacz et al. 2014; Arora 2018).

Importantly, increased cold tolerance is impacted by other environmental stressors and correlated with other plant tolerance and persistence traits. In particular, plant tolerances to different types of stress are known to co-vary (Vinebrooke et al. 2004; Lucas et al. 2013). For example, droughts can promote cold tolerance through a reduction in water content in leaf tissue and upregulation of certain enzymes (Medeiros and Pockman 2011). In addition, species that are more likely to occur in regularly burned grasslands tend to have thicker, sturdier leaves that are also more tolerant of cold (Ladwig et al. 2018). In addition to covarying stress tolerance traits, the timing of emergence and growth for individual species may shape how plants respond to freezing conditions. For example, early-emerging species may invest more in the ability to tolerate freezing temperatures since they are exposed to colder temperatures than late-emerging species (Pardee et al. 2019).

Although theoretically robust, few predictions about plant cold tolerance and survival strategies during early spring have been tested in natural herbaceous communities. This paucity of knowledge stems from a general lack of understanding cold tolerance and winter survival strategies for nonagricultural plant species (Rapacz et al. 2014; Geange et al. 2021). Measuring cold tolerance across many species and wide-ranging environmental conditions is daunting. In particular, determining when to measure cold tolerance and incorporating natural variability from not only climate but also disturbances, such as fire, is a key challenge. Yet assessing survival strategy tradeoffs amid the reality of natural fire regimes and changing winter conditions is critical for predicting plant species and community responses to climate change in temperate and polar regions and informing management actions to increase the resilience of grasslands and rangelands around the world.

Ecological disturbance regimes have served as an important selective force on plant life history strategies and may, therefore, set the stage for plant community responses to climate change (Johnstone et al. 2016). For example, temperate grasslands and rangelands produce large quantities of litter each year (Knapp and Seastedt 1986) that historically led to frequent, low-severity fires that supported diverse perennial plant communities with species characterized by traits that promote persistence and stress tolerance (Veldman et al. 2015). Also, grazing by various herbivores reduces plant biomass and acts as a natural disturbance. For sites that are too small to support mesograzer populations or where native grazers populations have declined, mowing can be used as a replacement management practice. In addition, fire is conducted through prescribed burning in the fall or spring seasons. These routine disturbances promote plant diversity by opening space for plant establishment and growth, reducing the dominance of grasses (Kelemen et al. 2014; Doležal et al. 2019), creating spatial and temporal heterogeneity, and moderating anthropogenic stresses from nutrient addition (Collins et al. 1998). Both fire and mowing remove litter with different effects, even when they are applied at the same time. Although both remove litter, fire will more thoroughly remove biomass, smoke can promote germination (Jefferson et al. 2008), and heat can volatilize nitrogen (Hobbs et al. 1991; Turner et al. 1997) and stimulate root production (Kitchen et al. 2009).

The timing of fire and mowing relative to winter, therefore, could amplify the effects of changing winter climate by determining if litter is present during winter. If fires or mowing occur in the fall, litter is removed before winter, reducing soil insulation (Lubbe and Henry 2019). Average winter soil temperatures decrease as a result, but soils thaw sooner in the spring (Groffman et al. 2001), allowing plants to emerge earlier. In contrast, spring fires allow for litter to overwinter so that soil temperatures during winter are warmer, yet soils thaw later in spring, leading to later plant emergence. Additionally, spring fires but may damage young tissues of plants emerging before burning occurs, thus favoring species that emerge later in the season (Towne and Craine 2014).

Here, we investigate how plant leaf investment strategies change in response to disturbance type and timing along with changing winter conditions in a temperate grassland. Specifically, we ask the following three questions:

1) How does disturbance type and timing affect specific leaf area (SLA) and leaf cold tolerance from the onset of spring through the growing season?
2) How do early spring leaf cold and stress tolerance traits relate to each other?
3) Are there tradeoffs between leaf growth and stress tolerance?

We predicted that regardless of disturbance type, fall treatments would promote earlier emergence and growth because the lack of litter over winter should promote earlier soil thawing. Additionally, plants in fall treatments would have leaves with the highest cold tolerance and lowest SLA because they are most prone to damage from cold or dry conditions. Since fall burning results in overwintering plants that are most exposed to harsh winter conditions, we predicted that plant responses would be stronger relative to fall mowing treatments where some leaf litter remains intact and can serve as an insulative barrier. We predicted that spring burning would result in leaves that have increased cold tolerance and lower SLA relative to control sites. Irrespective of our experimental treatments, we generally expected SLA would help predict leaf cold tolerance, where leaves with a lower SLA will be more tolerant of cold. Finally, we expected a tradeoff between growth and cold tolerance and between growth and SLA in leaves. Specifically, species that are more cold hardy or have leaves with lower SLA can emerge sooner but will grow more slowly due to the energetic cost of maintaining cold tolerance or building leaves with low SLA.

Methods

Experimental setup and sample collection

We established a field experiment in 2016 to manipulate disturbance type and timing in a restored tallgrass prairie in south central Wisconsin (42.9604 N, 89.8695 W). This region is characterized by a temperate climate with cold winters, but winters have warmed substantially since 1950 (Kucharik et al. 2010) and it is expected to receive less snow in the future (Notaro et al. 2011).

Table 1
Target species and their characteristics. Month of first flowering comes from the Online Virtual Flora of Wisconsin (Wisconsin State Herbarium, http://wisflora.herbarium.wisc.edu/index.php).

Species name	Growth form	Month of first flowering
Bromus inermis	Grass	June
Monarda fistulosa	Forb	July
Ratibida pinnata	Forb	June
Silphium integrifolium	Forb	July
Symphiotrichum laeve	Forb	August

The prairie where we conducted this study was restored in 2011 from corn agriculture using a diverse mix of forb and grass seeds and had been burned every 2–4 yr before our experiment. Eight replicate blocks were established in 2016 that each contained four 10 × 20 m plots randomly assigned to one of four disturbance treatment levels (fall burn, fall mow, spring burn, and control, Fig. S1, available online at …). Disturbances were applied annually from 2016 to 2019. All disturbance treatments were conducted by volunteers associated with Prairie Enthusiasts, the organization that owns and manages the field site. Prescribed fires were performed in late November or early December during the fall and in March during the Spring on days with low wind and relative humidity. Fires were ignited using a drip torch. As these are productive sites, prescribed fires were intense with flame heights that often exceeded 2 m and consumed > 90% of the standing litter. Mowing occurred in October each year and cut all plants at 10 cm. Cut biomass was left in the plot.

Starting in spring 2019, we collected leaf samples from each disturbance treatment plot seven times during the spring and early summer 2019 (April 15–June 20) for five common target spring species (four forbs and one grass). Target species were all perennial herbaceous species common across the site that emerge in spring but vary in growth form and flowering phenology (**Table 1**). The four forb species were seeded during the initial restoration and are now the common forb species, but the grass, *Bromus inermis,* was not seeded and instead likely dispersed from large areas of nearby land that was seeded with that species to promote topsoil conservation. While traditionally dominant warm-season grass species like *Andropogon gerardii* and *Sorghastrum nutans* are abundant in our plots, the timing of their growth initiation in the spring was much later than other species, so we did not include them in this study. On each collection day, we collected the largest, most developed leaf (including petiole but excluding grass sheaths) from a stem of each species in each experimental plot (4 treatment types • 8 experimental blocks = up to 32 leaves per species per sampling date). Not all species were located in each plot at each sampling date, so some species have fewer samples per sampling date (sample numbers per species on each date range from 1 to 32). Harvested leaves were wrapped in wet paper towels and placed in plastic bags in a cooler with ice packs until traits were measured in the laboratory that same afternoon (Pérez-Harguindeguy et al. 2013).

To assess the temperature conditions in each treatment, we measured the temperature of the air at 2 m and soil at 2 cm depth using iButton dataloggers (DS1921G-F5 Thermochron, 4K) every 2 h for the winter preceding sample collection. Dataloggers were deployed in 6–9 randomly selected plots of each treatment, and air dataloggers were placed under radiation shields. Dataloggers in spring-burn treatments were removed earlier than the rest of the dataloggers to allow for spring burn treatments to occur without damaging the dataloggers.

Trait measurements

We measured leaf area, cold tolerance, and SLA of each collected leaf and estimated leaf growth rates for each species. To measure leaf area, we flattened each leaf under a sheet of nonreflective glass and took a photo with a ruler for scale included in the photograph (Pérez-Harguindeguy et al. 2013). We used ImageJ (Schneider et al. 2012) to analyze each photo for leaf area.

To measure cold tolerance, we developed an efficient method for measuring many samples simultaneously. Our approach was based on using differential thermal analysis to measure the ambient temperature when ice nucleation occurs in leaf samples (Sakai and Larcher 1987). We made one hole-punch from each photographed leaf (0.64 cm diameter), avoiding main veins. Cold tolerance was measured by cooling the leaf punches on an aluminum tray in a circulating bath (PolyScience, Niles, IL, AP15R-40) from 5°C to −25°C at a rate of 10°C/h. The temperature of each sample was measured every second using a FLIR, A635sc, thermal camera to determine freezing timing (Neuner and Lichtenberger 2020; Zaragotas et al. 2016). We used differential thermal analysis to determine the temperature at which each leaf sample froze by comparing the temperature time-series of each sample to a control temperature measured on a piece of laboratory tape in the sample tray. Laboratory tape was used as a control because it has reflective properties that allow for temperature measurements that closely track the temperature of the cooling bath during the trial as measured by thermocouples ($r = 0.99$). We determined the freezing point of each sample from twenty-five 0.85 mm^2 pixels centered on the sample using an automated spike detection algorithm. The median value of the temperature when a spike occurred in the 25 pixels was used as the freezing temperature of each sample. This method made it possible to measure small samples from very young plants. We are also able to test ≈100 samples in 3 h, allowing us to measure many replicates to determine the importance of variation within species, between species, and through time.

To measure SLA, we dried each leaf punch from the freezing trial in a drying oven at 55°C for at least 72 h before measuring the dry mass of each sample. To estimate the growth rate of spring leaves, we calculated the total mass of each leaf that we sampled at each time point by dividing the leaf area of that leaf by the SLA. We then calculated the relative leaf growth rate through time for each species by calculating the slope of the log-transformed leaf mass through time.

Analysis

To assess how disturbance treatments affect leaf characteristics in the spring, we used linear mixed effect models to predict species traits as a function of species identity, collection time, disturbance treatment, and their interactions as fixed effects. To account for the repeated measures and the blocked design of this experiment, we allowed the intercept and slope through time for each experimental block, along with each plot nested in block, to vary randomly. We used this approach to assess how SLA changes through time and vary by species and disturbance type. To determine whether leaf cold tolerance changes through time and by species, we fit the same model as detailed earlier but also included freezing trial as a random intercept to account for potential unintended differences between each thermal recording. Both mixed effect models were fit using the “lmer” function from the “lmerTest” package in R (Kuznetsova et al. 2017).

We assessed whether traits were related to each other by using Pearson correlations among leaf area, SLA, and cold tolerance. We also assessed which factors best predict leaf cold tolerance by calculating partial r^2 values for disturbance treatment, species, leaf area, and SLA in explaining variation in cold tolerance using the “asbio” package in R (Aho 2020).

Finally, to assess whether there were leaf growth–tolerance tradeoffs in the species that we studied, we calculated leaf relative growth rates by using the slope of the log-transformed leaf

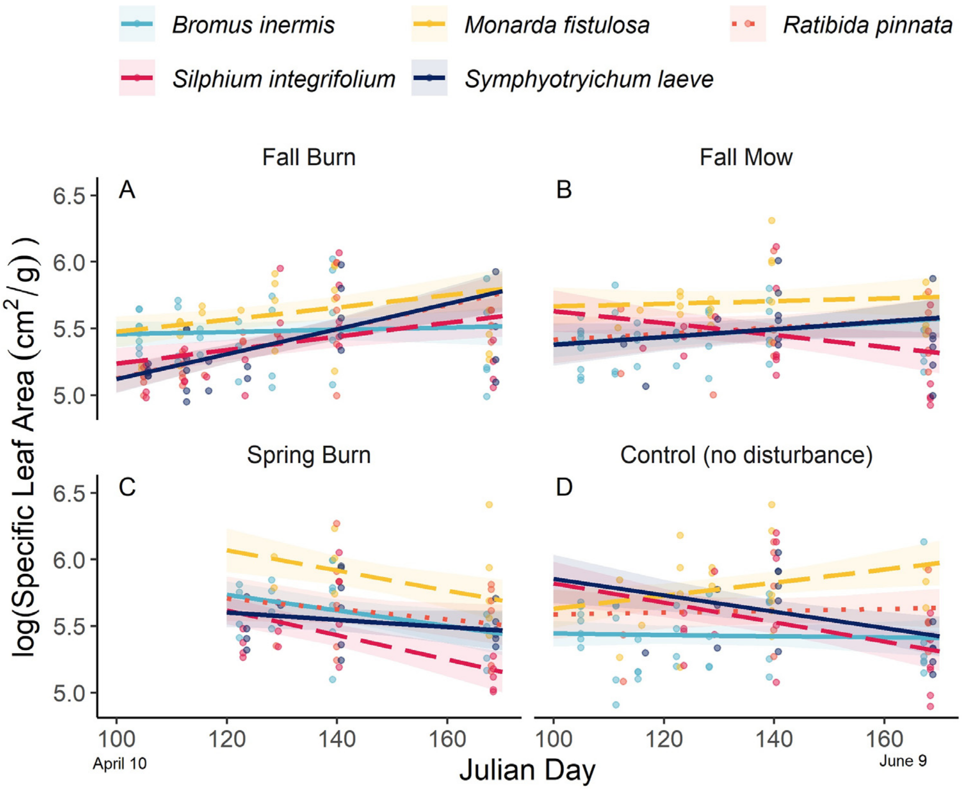

Fig. 1. **A–D,** Changes in specific leaf area over time for each species in disturbance treatments. Lines indicate best fits from a linear mixed effect model (± standard error).

mass through time for each species (from April 15 to June 20). We compared these growth rates with the average leaf tolerance traits (SLA and cold tolerance) for each species and assessed whether they were correlated using Pearson correlation tests. All analyses were performed in R (RCoreTeam 2017) and had an alpha of 0.05.

Results

Air and soil temperatures

Disturbance treatments affected soil temperatures as predicted. Fall burn treatments tended to result in the coldest soil temperatures at 2-cm depth during the winter months but the warmest soil temperatures following winter (Fig. S2, available online at …). Fall mow treatment plots were colder than control and spring burn plots but warmer than fall burn plots during the winter. Control and spring burn plots had similar soil temperatures throughout the experiment.

Disturbance effects on leaf traits through time (Question 1)

Both tolerance traits (SLA and cold tolerance) changed through time, but in response to different factors (**Table 2**, **Fig. 1**). For SLA, responses to disturbances depended on species identity. While fall burns tended to result in leaves with lower SLA and spring burns tended to result in leaves with higher SLA earlier in the season, responses to other disturbances (fall mow and control) varied by species (see Fig. 1). On the other hand, cold tolerance did not vary by species but increased through time in all treatments (lower freezing point), especially in spring burn treatments (**Fig. 2**).

Relationships among emerging leaf traits (Question 2)

Table 2
Analysis of variance table for models of tolerance traits as a function of disturbance treatment (fall mow, fall burn, spring burn, undisturbed control), species identity (see Table 1), and sampling time (day of year). Statistics reported include F (numerator df, denominator df) and significant *P* values (bold) where $^1 = P < 0.05$, $^2 = P < 0.01$, and $^3 = P < 0.001$. Degrees of freedom are estimated using the Satterthwaite method.

Term	Specific leaf area F value (d.f.)	Cold tolerance (freezing point) F value (d.f.)
Disturbance treatment	**4.7 (3, 18)**^{1}	**3.1 (3, 18.5)**^{1}
Species identity	1.7 (4, 340)	1.3 (4, 179)
Time	0.02 (1, 10)	**28.5 (1, 8.6)**^{3}
Disturbance:Species	**1.8 (12, 338)**^{1}	0.9 (12, 188)
Disturbance:Time	**3.6 (3, 17)**^{1}	**3.4 (3, 16)**^{1}
Species:Time	**2.4 (4, 341)**^{1}	1.4 (4, 180)
Species:Time:Disturbance	1.6 (12, 339)	1.1 (12, 185)

Leaf area, SLA, and cold tolerance were only weakly correlated with each other (**Fig. 3**). The strongest correlation was between freezing point and leaf area, as larger leaves tended to be more cold tolerant (see Fig. 3). Leaf area was also the strongest predictor of freezing point (partial $r^2 = 0.28$) when compared with SLA (partial $r^2 = 0.0001$), disturbance treatment (partial $r^2 = 0.01$), and species identity (partial $r^2 = 0.07$).

Growth-tolerance tradeoff in emerging leaves (Question 3)

There was weak evidence of a tradeoff between leaf relative growth rate and SLA (Pearson's $r = 0.79$, $P = 0.11$) where species that had, on average, lower SLAs grew slower (**Fig. 4A**). Likewise, there was some evidence of a tradeoff between cold tolerance and leaf relative growth rate (Pearson's $r = 0.81$, $p = 0.10$) where more cold-tolerant species also tended to grow slower (Fig. 4B).

Discussion

Disturbance timing affects growth and stress tolerance strategies in early spring for leaves of common grassland perennials. Burning in the fall results in emerging leaves that are more

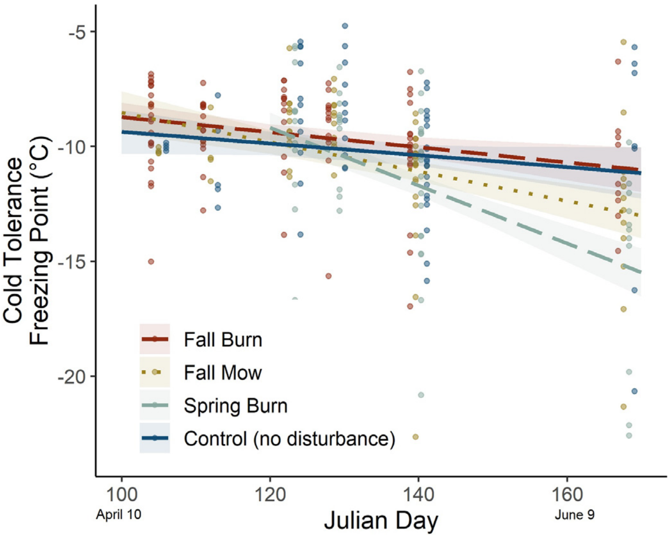

Fig. 2. Changes in cold tolerance (freezing point) for all species through time in different disturbance treatments. A lower freezing point indicates a more cold-tolerant tissue. Lines indicate fits from a linear mixed effect model (± standard error).

stress-tolerant (lower SLA, higher cold tolerance) while burning in the spring results in emerging leaves that are less stress-tolerant (higher SLA, lower cold tolerance; see Fig. 2). Furthermore, there were species-specific changes through time in SLA and some evidence for a species-level tradeoff between growth and tolerance.

The effect of fire season on the characteristics of early emerging leaves could set the stage for plant growth strategies over the entire growing season. In our experiment, the SLA of individuals in spring-burned plots declined over time, in line with other ontogenetic studies (Mason et al. 2013; Henn and Damschen 2021). In fall-burned plots, however, individuals had lower SLAs early in the growing season that increased over time, becoming similar to other disturbance treatments later in the season (see Fig. 1). Since leaves in fall-burned plots are emerging from the most extreme winter conditions, this pattern suggests that plants burned in the fall may adopt a more conservative growth strategy during early growth. This difference in strategy could be caused by several mechanisms. Because our treatments occurred over 3 yr, longer than the estimated lifespans of many perennial herbaceous grassland species (Lauenroth and Adler 2008), the differences in responses are unlikely due to selection, but instead to phenotypic plasticity. Since the plants that we sampled had been exposed to the same disturbance treatments for several years, the plants in each treatment could have acquired epigenetically based adaptations to the differences in temperature or other environmental conditions caused by differences in disturbance timing (He and Li 2018). Alternatively, the timing of spring emergence and growth depends on the timing of soil thaw and temperature cues for growth initiation (Lubbe et al. 2021) and since the timing of disturbance influences winter and spring soil temperatures, plants in fall burn plots likely deacclimate to cold temperatures sooner and are, thus, in a more advanced stage of development than plants that deacclimate later.

Despite differences in SLA in early-season leaves, the cold tolerance of leaves did not differ between species or among disturbance treatments until later in the spring. The lack of species-level differences in cold tolerance indicates the importance of environmental conditions in determining tissue cold tolerance levels. However, other studies have found minimal to no associations between leaf cold tolerance and environmental conditions (Wheeler et al. 2014). Leaves in spring burn plots became more cold hardy through time (see Fig. 2), which was surprising, as warming spring temperatures typically result in plant deacclimation and leaves that are less cold tolerant (Li et al. 2002; Zuther et al. 2015). Relatively consistent cold tolerance levels for most disturbances through time indicate that plants had deacclimated before sampling. The unexpected results in spring burn plots might also suggest that seasonal cold tolerance patterns may be a result of correlations with other functional traits like leaf area (Ladwig et al. 2018) or environmental conditions like drought (Medeiros and Pockman 2011). Overall, the range of cold tolerance observed for the perennial herbaceous plants in our study was similar to previous studies (Bauer et al. 1994), tolerating cold between −5°C and −15°C.

Compared with plots burned in the fall or spring, mowing in the fall tended to dampen leaf responses. This is likely because, compared with burning in the fall, mowing disrupts litter structure rather than removing litter biomass. The impacts of fall mowing and fall burning treatments, however, were often similar, suggesting that litter reduction and compaction from mowing has a similar but smaller effect on plant responses as complete litter removal from burning. This is likely due to the important role of litter depth in insulating the soil and determining plant emergence and growth (Lubbe and Henry 2019). It is, however, important to note that mowing is unlikely to have exactly the same effects as grazing because grazing will result in differential plant consumption based on grazer preferences (Steuter and Hidinger 1999).

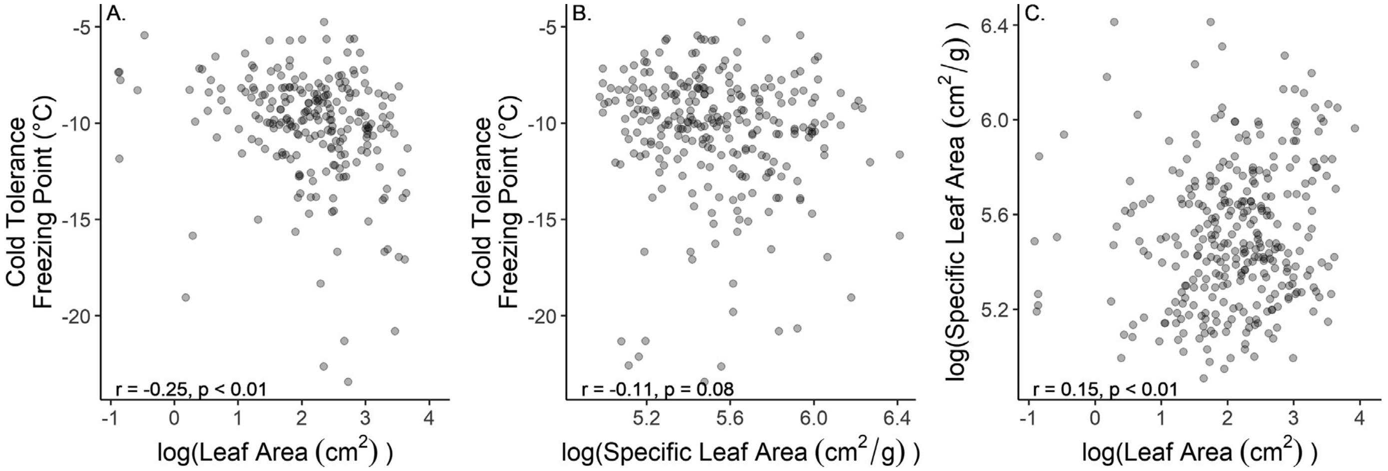

Fig. 3. Correlations between leaf traits. **A–C,** Text in each panel indicates the Pearson correlation coefficient for the correlation between the two traits in each panel regardless of treatment type.

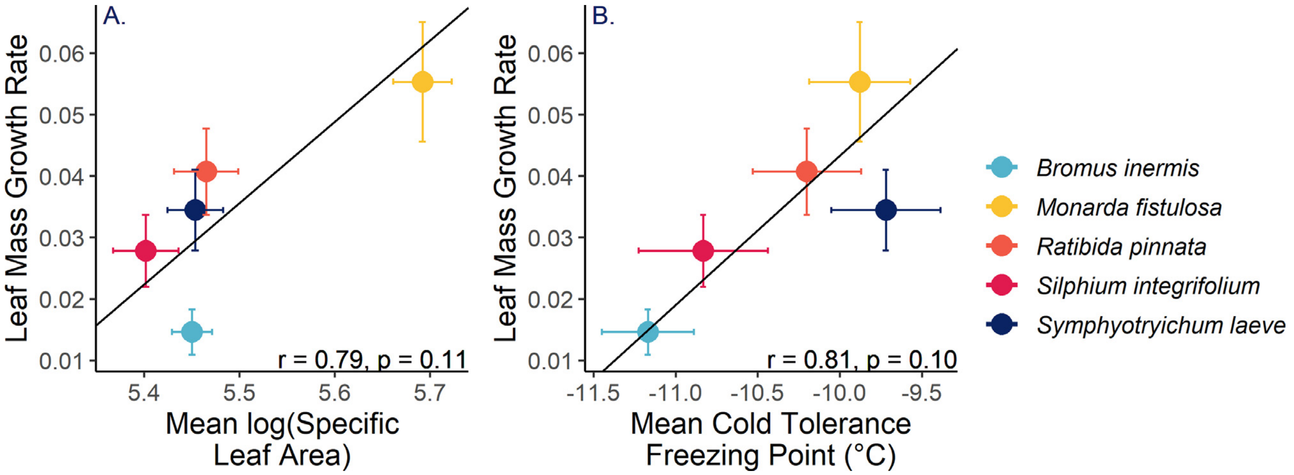

Fig. 4. Relationship between (**A**) log mean specific leaf area and leaf mass growth rates and (**B**) mean leaf cold tolerance (more negative values indicate greater cold tolerance) and leaf mass growth rates (± standard error) for the five study species. Lines indicate standard major axis regression lines and Pearson's correlation coefficients along with *P* values are reported within each panel.

The expected growth-tolerance tradeoff between rapid growth and the ability to tolerate stress (Koehler et al. 2012) was partially supported in young, emerging leaves. For both freezing point and SLA, more tolerant leaves showed a trend toward growing more slowly (**Fig. 4**), but this was not statistically significant. Although our data did not reveal a strong growth-tolerance tradeoff, this could be because our measures of leaves come from a variety of environmental contexts that capture other local processes influencing trait variation or because we studied a limited number of species. Species often trade off the ability to tolerate, resist, or avoid cold on the basis of differential risks of damage and abilities to use energy for growth (Agrawal et al. 2004). In other words, plants can accept the risk of damage to tissues and mitigate damage by having high tolerance of cold (i.e., cold resistance/tolerance), or they can avoid damage altogether by remaining dormant underground until cold temperatures have most likely passed. The former strategy has the advantage of a longer growing season but the disadvantage of investing more energy into stress tolerance at the expense of growth.

We hypothesized that there would be some variation in cold-tolerance strategies among species with different flowering and emergence phenologies. For example, we expected that earlier-flowering species should possess greater cold tolerance (a cold-resistance strategy) rather than adopt an avoidance strategy of staying below ground or a strategy of accepting spring damage because their fitness depends on early growth while preventing tissue damage. We see some evidence for this hypothesis from our study where the earliest-flowering species *(Bromus inermis)* was also the most cold tolerant while the latest-flowering species *(Symphyotrichum laeve)* was the least cold tolerant. However, growth requires cold deacclimation (Ma et al. 2019), so early-emerging species are exposed to greater frost risk. For example, both *Monarda fistulosa* and *B. inermis* produce new leaves in the fall that overwinter above ground. This strategy allows for early nutrient and light capture in the spring, but the leaves must survive cold winter conditions. *B. inermis* leaves were almost fully expanded before our study began, and we observed some frost damage while collecting tissues, indicating that the risk of early damage is outweighed by the advantage of early growth for that species. *M. fistulosa,* on the other hand, did not show similar damage, but the overwintering leaves were small and did not begin to grow until temperatures were warmer in the spring. On the other hand, the dominant C4 grasses such as *Andropogon gerardii* and *Panicum virgatum* in prairies emerge much later in the spring but appear to be more sensitive to cold damage, experiencing noticeable damage at temperatures of only −3°C (Mitchell and Redfearn 2019). There may be a life history spectrum where species adopt a strategy of either emerging (and flowering) early with high cold tolerance and resistance or emerging (and flowering) late and avoiding cold temperatures altogether (**Fig. 5**). Further characterizing these life history dimensions could have important implications for predicting how species will respond to changing winter conditions along with disturbance and management regimes.

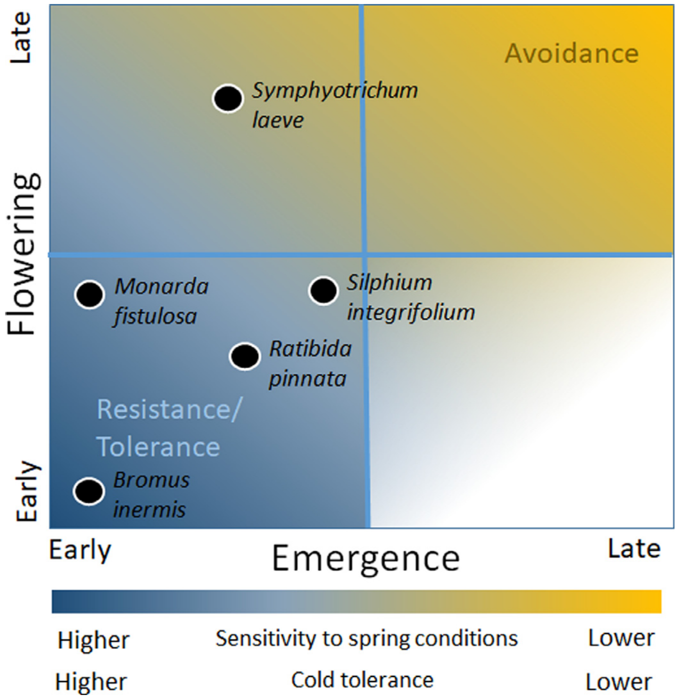

Fig. 5. Hypothesized gradient in sensitivity to cold conditions during the spring and how it may depend on emergence and flowering timing in perennial herbaceous plants.

Implications

Overall, disturbance type and timing affect how plants invest energy in growth and cold tolerance across the growing season. Burning in the fall results in individuals with leaves that have lower SLAs earlier in the season, while burning in the spring results in individuals with leaves that become more cold tolerant through time. Mowing in the fall results in individuals with similar leaf characteristics to those in unburned or spring-burned plots. Furthermore, the associations between functional traits demonstrate fundamental tradeoffs between growth and stress tolerance that can lead to changes in community composition under different environmental conditions. As the climate changes in temperate grasslands and rangelands, altered spring temperatures and extreme events may favor species on either side of this growth-tolerance tradeoff, depending on the type and timing of disturbance. These results suggest that land managers may promote individuals with more stress-tolerant strategies during early spring by burning in the fall. On the other hand, burning in the spring will promote individuals with a faster growth strategy while disfavoring individuals with higher stress tolerance in the early spring. Because plant strategy responses to disturbance timing are different depending on disturbance timing, applying managed disturbance at a variety of times is likely to favor the largest variety of species and strategies and promote resilience to climate change.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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