

# Greater bud outgrowth of *Bromus inermis* than *Pascopyrum smithii* under multiple environmental conditions

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## Abstract

### Aims

Tiller recruitment of perennial grasses in mixed-grass prairie primarily occurs from belowground buds. Environmental conditions, such as temperature, soil moisture and grazing can affect bud outgrowth of both invasive and native perennial grasses. Differential bud outgrowth responses of native and invasive species to climate change and grazing could alter competitive interactions that have implications for future land management. The aims of this work were to (i) compare how spring temperature altered bud outgrowth of native *Pascopyrum smithii* (Rydb.) Á. Löve (western wheatgrass) and introduced *Bromus inermis* Leyss. (smooth brome), (ii) compare how watering frequency altered bud outgrowth of these two species and (iii) evaluate how clipping interacts with spring temperature or watering frequency to affect *P. smithii* bud outgrowth.

### Methods

Individual plants of *B. inermis* and *P. smithii* were harvested from North American mixed-grass prairie. Bud outgrowth from tillers of both species were evaluated under three spring temperature regimes (Average: 12, 18 and 24°C) and two watering frequencies (frequent and intermittent) in a growth chamber experiment. The response of *P. smithii* bud outgrowth to clipping was also examined.

### Important Findings

*Bromus inermis* had more buds per tiller and initiated a greater proportion of these buds than *P. smithii* under all temperature and moisture conditions. *Pascopyrum smithii* bud development was reduced at 24°C. Intermittent watering did not significantly impact bud outgrowth of either species. Clipping increased *P. smithii* bud mortality and reduced its bud development for the 2-week period of the study. The robust vegetative reproductive capacity of *B. inermis* under a range of environmental conditions is a key mechanism enabling the expansion of *B. inermis* into *P. smithii*-dominated mixed-grass prairie in North America. Mixed-grass prairie dominated by *P. smithii* experiencing repeated defoliation may require longer recovery times and be more susceptible to *B. inermis* invasion due to the negative impact of grazing on *P. smithii* bud outgrowth. Successful tiller recruitment and establishment of native perennial grasses via the bud bank will be necessary for mixed-grass prairie to be resilient to climate change, plant invasions and grazing.

**Keywords:** bud bank, climate change, drought, grazing, invasive species, perennial grass

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## INTRODUCTION

Seedling recruitment of perennial grasses is rare as most tillers are recruited from vegetative belowground buds (Benson and Hartnett 2006; Fair *et al.* 1999; Peters 2000; Rogers and Hartnett

2001). The belowground bud bank (*sensu* Harper 1977) maintains a critical role in grasslands by providing resilience and long-term persistence of perennial grasses in a fluctuating environment. Bud banks are a clonal trait that influence grassland assembly, structure and response to grazing, fire and short-term

drought (Dalglish and Hartnett 2009; Klimesova and Klimes 2008; Rusch et al. 2011; VanderWeide and Hartnett 2015). Bud bank demography, including bud production, longevity and outgrowth, is influenced by both current and past precipitation, which can create a legacy effect on grassland aboveground net primary production (ANPP; Ott and Hartnett 2012; Reichmann and Sala 2014; Reichmann et al. 2013). If previous-year precipitation was high and produced a high bud bank density, ANPP could greatly increase in a subsequent wet year (Dalglish and Hartnett 2006; Knapp and Smith 2001). Because of their sensitivity to environmental conditions, bud banks of grassland biomes may be one of the most responsive population and community-level traits to environmental change.

Temperature, CO<sub>2</sub> concentrations and inter-annual variability in precipitation are predicted to increase in the North American Great Plains (IPCC 2007; Kunkel et al. 2013; Patricola and Cook 2013). Climate change studies have mostly examined the physiological and production responses of plants to elevated CO<sub>2</sub>, temperatures and altered moisture regimes (e.g. Morgan et al. 1994; Read and Morgan 1996; Yu et al. 2012; Zelikova et al. 2014). Tiller production, especially of C<sub>3</sub> grasses, increased under elevated CO<sub>2</sub> (Ward et al. 1999), but more studies are needed to examine the demographic plant response via the bud bank. Under several future climate scenarios, buds will be exposed to a wider range of conditions and their response will determine how grassland production and community composition will be altered.

In addition to climate change, northern C<sub>3</sub>-dominated native plant communities of the Great Plains face the threat of invasion by introduced C<sub>3</sub> perennial grasses, including *Bromus inermis* Leyss. (smooth brome; Christian and Wilson 1999; DeKeyser et al. 2013; Larson et al. 2001). Transformation of large tracts of native grasslands to *B. inermis* monocultures has reduced native plant diversity (Dillemuth et al. 2009; Frank and McNaughton 1992) and habitat use by native ungulates (Trammell and Butler 1995). *Bromus inermis* readily outcompeted its native neighbors in northern mixed-grass prairie even under drought conditions (Nernberg and Dale 1997; Ulrich and Perkins 2014). Although *B. inermis* has high rates of seedling establishment (Ries and Hofmann 1996) and facilitates its invasion via soil modifications (Jordan et al. 2008), *B. inermis* expansion and success largely depends on its vegetative proliferation via belowground buds and rhizomes and its resulting integration among ramets (Otfinowski and Kenkel 2008; Otfinowski et al. 2007).

Climate change has the potential to differentially affect the reproduction and growth of native and non-native C<sub>3</sub> perennial grasses, such as the native *Pascopyrum smithii* (Rydb.) Á. Löve (western wheatgrass) and the non-native *B. inermis* in North America. *Bromus inermis* seedling establishment and survival was lower when the ambient temperature was elevated by 0.3°C (Sheppard et al. 2012). However, *B. inermis* maintained peak biomass production over a wider range of temperatures than the native western wheatgrass under well-watered conditions in a growth chamber experiment (Morrow and Power 1979). *Pascopyrum smithii* is a clear dominant in semi-arid mixed-grass

prairies of the northwestern Great Plains, where it can appear as a dense monoculture, but its climate tolerance relative to *B. inermis* is unclear. Differential bud outgrowth responses of *B. inermis* and *P. smithii* to extreme variations in precipitation and temperature associated with climate change could affect the outcome of their future competitive interactions.

Although *P. smithii* is a major constituent in many semi-arid grasslands, information on how defoliation interacts with moisture and temperature to influence bud bank dynamics of *P. smithii* is lacking (but see Ott and Hartnett 2015b). In mesic tallgrass prairie, grazing increased the tillering rate from the grass bud bank resulting in lowered bud bank densities that decreased the capacity of the grassland to respond to resource pulses (Dalglish and Hartnett 2009). If defoliation stimulated bud outgrowth of *P. smithii*, survival of these new tillers under conditions of drought and higher temperatures will likely be reduced leading to a decline in population size contributing to a decrease in ANPP (Dalglish and Hartnett 2009). Alternatively, low soil moisture and high temperatures could limit tiller recruitment following defoliation and preserve a large dormant bud bank prepared to respond when environmental conditions improve. Increased bud dormancy or mortality of *P. smithii* following defoliation may provide introduced grasses, such as smooth brome, opportunities to invade native grasslands.

Projected perennial grass production of northern mixed-grass prairie under climate change requires examination of the demographic response via the bud bank of native and introduced C<sub>3</sub> perennial grasses to changes in temperature and water availability, especially during tiller recruitment in the spring and fall and under grazing. Our main objectives were to (i) compare how spring temperature altered bud outgrowth of native *P. smithii* and introduced *B. inermis*, (ii) examine how watering frequency altered bud outgrowth of these two species and (iii) evaluate how clipping interacts with spring temperature or watering frequency to affect *P. smithii* bud outgrowth.

We hypothesized:

- 1) The invasive species (*B. inermis*) would have a larger bud supply and greater bud outgrowth than the native species (*P. smithii*).
- 2) Both species would exhibit higher bud mortality and lowered bud outgrowth at higher temperatures.
- 3) Less frequent rainfall would lower bud outgrowth but not increase bud mortality, especially in the native species.
- 4) Clipping would stimulate *P. smithii* bud outgrowth at low temperatures and consistent soil moisture simulating frequent rainfall.

## METHODS

### Collection site and sampling

The Buffalo Gap National Grassland is a 201 200-ha mixed-grass prairie managed by the US Forest Service in western South Dakota. The region's semi-arid climate has cool winters

(average January temperature:  $-4.9^{\circ}\text{C}$ ) and warm summers (average July temperature:  $22.7^{\circ}\text{C}$ ) with moderate rainfall (425 mm) primarily occurring April through October, especially in May and June. Its vegetation is dominated by  $C_3$  native perennial grasses, such as *P. smithii*, *Hesperostipa comata* (Trin. & Rupr.) Barkworth, and *Nassella viridula* (Trin.) Barkworth, but includes many forbs and  $C_4$  grasses.

*Pascopyrum smithii* and *B. inermis* are strongly rhizomatous perennial  $C_3$  grasses that begin flowering in late May. Both species are abundant in the Northern Great Plains. *Pascopyrum smithii* is native to North America, but *B. inermis* has been introduced from Eurasia and spreads from seeded pastures and roadways.

At the end of May 2014, 350 individual plants of *P. smithii* and 175 individual plants of *B. inermis* were collected from a pasture near the French Creek Picnic Area in the Buffalo Gap National Grassland ( $43^{\circ}30'\text{N}$ ,  $103^{\circ}1'\text{W}$ ). Cattle had been excluded from the pasture in the previous and current growing season. Due to the rhizomatous growth form and intermingling of different genets within both species, genets are difficult to identify in the field. Therefore, an 'individual' of *P. smithii* or *B. inermis* consisted of all interconnected tillers and associated belowground parts within a 5.0 cm radius and to a depth of 15 cm. *Pascopyrum smithii* was uniformly distributed throughout the pasture. Therefore, parallel 50-m transects were placed every 10 m within the pasture and the nearest *P. smithii* individual was harvested every 3 m along each transect until 350 individuals were collected. Ten discrete clones of *B. inermis* were located throughout the pasture. Each clone was assumed to belong to the same genet because each clone was surrounded by native vegetation and typically <5 m in diameter. The number of individuals collected from each clone was roughly proportional to the size of the clone and no >40 individuals were collected from each clone ( $18 \pm 3$  average number of individuals collected per clone). Individuals were placed with their undisturbed native soil into 10-cm diameter peat pots and subsequently placed in a gallon plastic bag. All individuals were kept at  $4^{\circ}\text{C}$  in dark storage until they were placed in the growth chamber.

### Experimental setup

Four experiments were conducted to examine various treatment combinations involving species (*B. inermis* and *P. smithii*), temperature regime (low, medium and high), watering frequency (frequent and intermittent) and clipping (unclipped and clipped).

#### Experiment 1: temperature and species

Experiment 1 examined the interaction between temperature and species of unclipped plants with a frequent watering regime.

#### Experiment 2: watering frequency and species

Experiment 2 examined the interaction between watering frequency and species of unclipped plants at a low temperature regime.

#### Experiment 3: temperature and clipping effects on *P. smithii*

Experiment 3 examined the interaction between temperature and clipping of *P. smithii* with a frequent watering regime.

#### Experiment 4: watering frequency and clipping effects on *P. smithii*

Experiment 4 examined the interaction between watering frequency and clipping of *P. smithii* at a low temperature regime.

The four experiments were conducted concurrently during six 2-week trials using two growth chambers [Conviron CMP5000]. Three temperature regimens were evaluated: Low ( $10^{\circ}\text{C}$  night/ $16^{\circ}\text{C}$  day), Medium ( $16^{\circ}\text{C}$  night/ $22^{\circ}\text{C}$  day) and High ( $22^{\circ}\text{C}$  night/ $28^{\circ}\text{C}$  day). Two temperatures were examined per trial, one in each chamber (Table 1). In each trial, the two temperatures were assigned to the chambers insuring that each temperature in the overall experiment was run twice in each chamber. Each pair of temperatures was assigned to two trials insuring that each pair of temperatures occurred once in the first three trials and once in the last three trials. Therefore, each pair was examined in a block that required samples to spend fewer days in cold storage and a block that required samples to spend more days in cold storage (Table 1). Over the 14 h 30 min day length, temperatures ramped up to their peak daytime temperature in 6 h 30 min and then were maintained for 3 h before ramping down to their overnight temperature. These regimens and photoperiod were designed to reflect the spring daytime–nighttime temperature oscillations of soil rather than air as buds are more affected by soil temperatures due to their location on the plant. Average daily temperatures for the low, medium and high regimens were 12, 18 and  $24^{\circ}\text{C}$ , respectively. Light quantity ( $440 \pm 8 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the middle of the growth chamber; Decagon Devices; PAR Photon Flux Sensor: Model QSO-S) and humidity (60–80%; Essick Air 5D6 700) were controlled across all trials.

Within each growth chamber, a set of species, watering frequency and clipping treatment combinations were evaluated. The set of treatment combinations depended on the

**Table 1:** experimental setup of growth chambers

Trial	Chamber 1	Chamber 2	Days spent in cold storage
1	Low	Medium	34–40
2	High	Low	52–58
3	Medium	High	70–76
4	Medium	Low	87–93
5	Low	High	101–107
6	High	Medium	118–123

Each temperature regime (low, medium and high) was run twice in each growth chamber. Analyses were blocked by trial to control for effects of time spent in storage on bud outgrowth. Sample harvest from the field occurred over 7 days, which affected the amount of time each sample spent in cold storage. The range of days spent in cold storage for samples used in each trial is provided.

temperature being run in the growth chamber (Table 2). For a given temperature, each treatment combination was randomly assigned to eight replicate 10.2-cm diameter plastic pots. Individuals harvested in May 2014 were removed from cold storage and washed to remove their soil before the start of each growth chamber trial (Table 1). At least two photosynthesizing tillers were planted within each pot filled with pre-mixed soil (three parts field clay soil: one part sand, bulk density:  $0.80\text{ g cm}^{-3}$ ) insuring that individuals from a solitary clone or transect were not over-represented in any one replicate set of pots. If one individual did not have two live tillers, tillers from a second individual were used.

For the watering frequency treatment, pots were saturated with 150 ml water on the first day of the trial and allowed to dry down for 24–36 h until they had dropped below 40% volumetric water content (VWC; Decagon Devices; Soil Moisture Sensor: Model EC-5 custom calibrated to the soil). At this time, a frequent or intermittent watering frequency treatment was imposed on each pot. Frequently watered pots were watered twice daily which maintained adequate soil moisture for growth (i.e. VWC between 35% and 45%). Intermittently watered pots were watered every 4 days with the same total amount of water that frequently watered pots received over the previous 4 days. Intermittently watered pots experienced a dry down period reaching ~20–22% VWC followed by a rapid saturation event. Soil moisture sensor monitoring of VWC during each trial ensured maintenance of VWC within its pre-determined range. All water used underwent reverse osmosis. Clipping treatments consisted of clipped or unclipped plants. Clipped plants were cropped to a 4 cm height to simulate ungulate grazing height (Pfeiffer and Hartnett 1995). After being assigned a treatment, pots were randomly arranged within the growth chamber.

### Bud classification

At the end of each trial, buds and rhizomes of each individual plant were examined using a dissecting scope with magnifications between 6× and 25×. Rhizomes, basal/belowground

**Table 2:** treatment combinations applied within growth chambers with different temperature regimes

	<i>B. inermis</i> — Unclipped	<i>P. smithii</i> — Unclipped	<i>P. smithii</i> — Clipped
Low temperature			
Frequent	X	X	X
Intermittent	X	X	X
Medium and High temperature			
Frequent	X	X	X
Intermittent			

Each temperature regime (low, medium and high) had a specific set of species, moisture (frequent or intermittent watering regime) and clipping treatments that occurred within the growth chamber. An 'X' indicates that the treatment combination occurred at the specified temperature.

buds and new tillers borne on photosynthesizing tillers were counted, assessed to be living or dead and classified by size (Table 3).

Tillers and rhizomes were distinguished from buds by their elongation in relation to the prophyll. Buds were contained within the prophyll and tillers and rhizomes had elongated past the prophyll. Dead buds were identified by their soft, spongy or mealy brown interiors and easily distinguished from live buds. Collectively, all live buds, rhizomes and tillers growing from parent tillers were called 'live propagules'. Live propagules belong to the same cohort of buds.

### Statistical analysis

For each experiment, two response variables were analyzed at the completion of each growth chamber trial: live propagules per tiller and the proportion of live propagules in four developmental stages (B, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>).

#### Live propagules per tiller

To investigate temperature and species effects (Exp. 1), the number of live propagules was evaluated using a two-way factorial treatment structure with the factors of temperature (three levels) and species (two levels) in a balanced incomplete block design (BIBD) blocking on trial (six blocks) with a split-plot using a negative binomial distribution (PROC GLIMMIX, SAS Institute 2012). The factor of temperature was applied to each chamber within each trial and the factor of species was applied to each pot. To investigate temperature and clipping effects on *P. smithii* (Exp. 3), the same design structure and methods for Experiment 1 were used but the treatment structure was a two-way factorial with factors of temperature (three levels) and clipping (two levels). The factor of clipping was applied to each pot.

**Table 3:** bud, rhizome and tiller developmental stages

Symbol	Developmental stage	Description
B	Buds	Contained within the prophyll
T <sub>1</sub>	Small juvenile tillers/rhizomes	Apex elongated <3.0 mm past prophyll
T <sub>2</sub>	Large juvenile tillers/rhizomes	Vertically elongated >3.0 mm past prophyll AND <4.5 cm in total height ( <i>P. smithii</i> ) <3.6 cm in total height ( <i>B. inermis</i> ) OR Horizontally elongated >3.0 mm past prophyll
T <sub>3</sub>	Adult tillers	>4.5 cm vertical height ( <i>P. smithii</i> ) >3.6 cm vertical height ( <i>B. inermis</i> )

Tillers were considered juvenile tillers when ≤9% of the recorded vegetative tiller height for each species (Great Plains Flora Association 1986; Ott 2014).



To investigate soil moisture and species effects at the lowest temperature (Exp. 2), the number of live propagules was evaluated using a two-way factorial treatment structure with the factors of soil moisture (two levels) and species (two levels) in a randomized complete block design (RCBD) blocking on trial (four blocks) using a negative binomial distribution (PROC GLIMMIX, SAS Institute 2012). To investigate soil moisture and clipping effects on *P. smithii* at the lowest temperature (Exp. 4), the same design structure and methods for Experiment 2 were used but the treatment structure was a two-way factorial with factors of soil moisture (two levels) and clipping (two levels).

For each analysis involving the response variable of live propagules per tiller, the number of live propagules per pot was offset by the number of tillers within the pot (i.e. live propagules per tiller). Kenward-Roger's (KR) method was used to approximate the denominator degrees of freedom. Model goodness-of-fit was checked by insuring the deviance was at or near 1.

#### Proportion of live propagules at each developmental stage

For experiment 1, the proportion of live propagules belonging to each successive developmental stage was assessed using a two-way factorial treatment structure with the factors of temperature (three levels) and species (two levels) in a BIBD blocking on trial (six blocks) with a split-plot using a multinomial ordinal logit model assuming proportional odds (PROC GLIMMIX, SAS Institute 2012). A similar model was used for Experiment 3, but the two-way factorial treatment structure had the factors of temperature (three levels) and clipping (two levels).

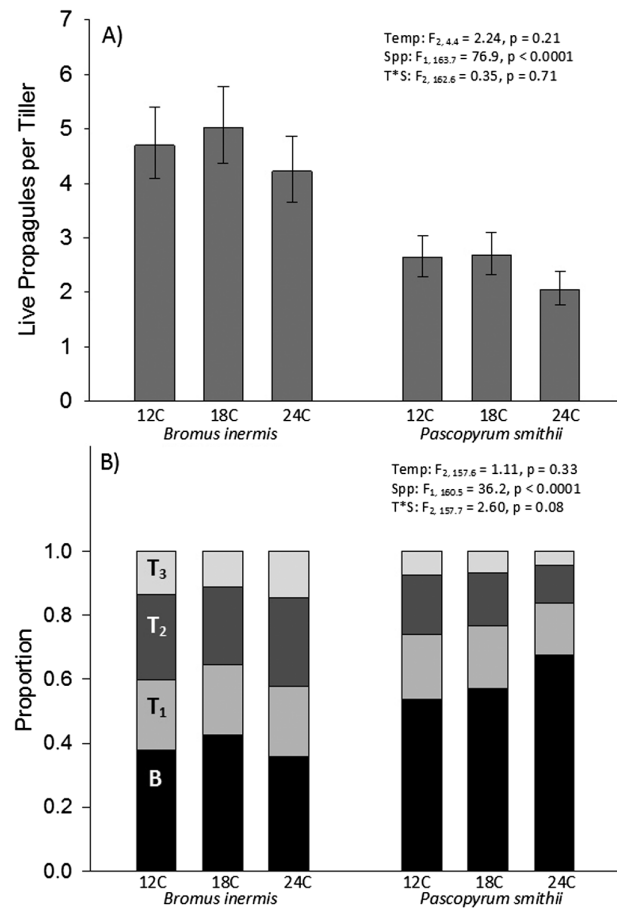
For Experiment 2, the proportion of live propagules belonging to each successive developmental stage was assessed using a two-way factorial treatment structure with the factors of soil moisture (two levels) and species (two levels) in a RCBD blocking on trial (four blocks) using a multinomial ordinal logit model assuming proportional odds (PROC GLIMMIX, SAS Institute 2012). A similar model was used for Experiment 4, but the two-way factorial treatment structure had the factors of soil moisture (two levels) and clipping (two levels).

For all experiments examining the proportion of live propagules at each developmental stage, denominator degrees of freedom were approximated using KR. Model estimates of proportions were used in the figures.

## RESULTS

### Experiment 1: temperature and species

The number of live propagules per tiller was unaffected by temperature but differed significantly between species (Fig. 1A). *Bromus inermis* maintained a greater number of propagules per tiller than *P. smithii*. Propagule development was significantly greater for *B. inermis* than *P. smithii* as greater proportions of live propagules were at higher developmental stages for *B. inermis* (Fig. 1B). The odds of a propagule being at a higher developmental stage (e.g. a tiller or rhizome ( $T_1$ ,  $T_2$ ,  $T_3$ ) versus a bud

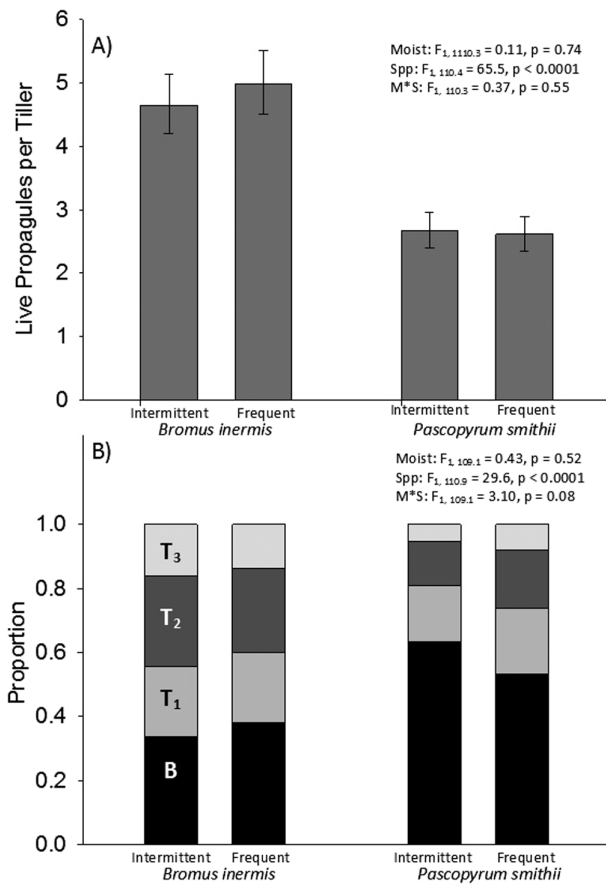


**Figure 1:** (A) live propagules per tiller and (B) propagule development response to temperature for *Bromus inermis* and *Paspopyrum smithii* under frequent watering conditions. The proportion of live propagules were classified into four developmental/size classes including buds (B), small juvenile tillers/rhizomes ( $T_1$ ), large juvenile tillers/rhizomes ( $T_2$ ) and adult tillers ( $T_3$ ; see Table 1 for more detailed descriptions). Error bars  $\pm 1$  SE based on the statistical model.

(B)) was 2.35 $\times$  greater for *B. inermis* propagules than *P. smithii* propagules (odds ratio [OR] 95% confidence interval [CI] [1.77, 3.11]). Because the proportional odds assumption was met by the model, the odds of a propagule being an adult tiller ( $T_3$ ) versus a juvenile tiller, rhizome or bud (B,  $T_1$ ,  $T_2$ ) or the odds of a propagule being a large juvenile tiller, rhizome or adult tiller ( $T_2$ ,  $T_3$ ) versus a small juvenile tiller or bud (B,  $T_1$ ) were also 2.35 $\times$  greater for *B. inermis* than *P. smithii*. The effect of temperature on propagule development differed slightly by species (Fig. 1B). Propagule development of *B. inermis* remained unchanged across all three temperatures, but the highest temperature had a marginally significant reduction in propagule development for *P. smithii* when compared to the lower two temperatures.

### Experiment 2: watering frequency and species

Intermittent watering did not affect the number of live propagules per tiller for either species (Fig. 2A). However, similar to Experiment 1, *B. inermis* maintained  $\sim 2\times$  the number of buds

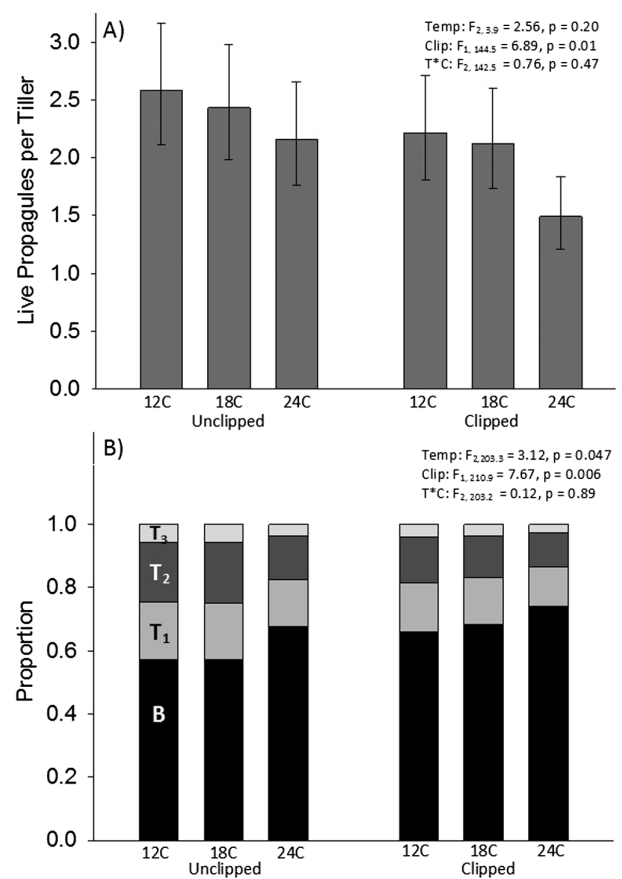


**Figure 2:** (A) live propagules per tiller and (B) propagule development response to watering frequency treatments for *Bromus inermis* and *Paspopyrum smithii* at 12°C. The proportion of live propagules were classified into four developmental/size classes including buds (B), small juvenile tillers/rhizomes ( $T_1$ ), large juvenile tillers/rhizomes ( $T_2$ ) and adult tillers ( $T_3$ ; see Table 1 for more detailed descriptions). Error bars  $\pm 1$  SE based on the statistical model.

per tiller as did *P. smithii*. Propagule development was significantly greater for *B. inermis* than *P. smithii* (Fig. 2B; OR = 2.51 with 95% CI [1.80, 3.50]). The effect of intermittent watering on propagule development differed slightly by species (Fig. 2B). For *P. smithii*, propagule development tended to be lower in the intermittent frequency treatment, whereas propagule development remained unchanged between watering treatments of *B. inermis*.

### Experiment 3: temperature and clipping for *P. smithii*

Clipped tillers had significantly lower numbers of propagules than unclipped tillers (~0.5 fewer propagules per clipped tiller; Fig. 3A). Temperature did not affect the number of propagules per tiller. Propagule development was significantly lower for clipped plants and significantly differed by temperature (Fig. 3B). The odds of a propagule being at a higher developmental stage were 1.47× greater for unclipped as compared to clipped *P. smithii* propagules. For clipped and

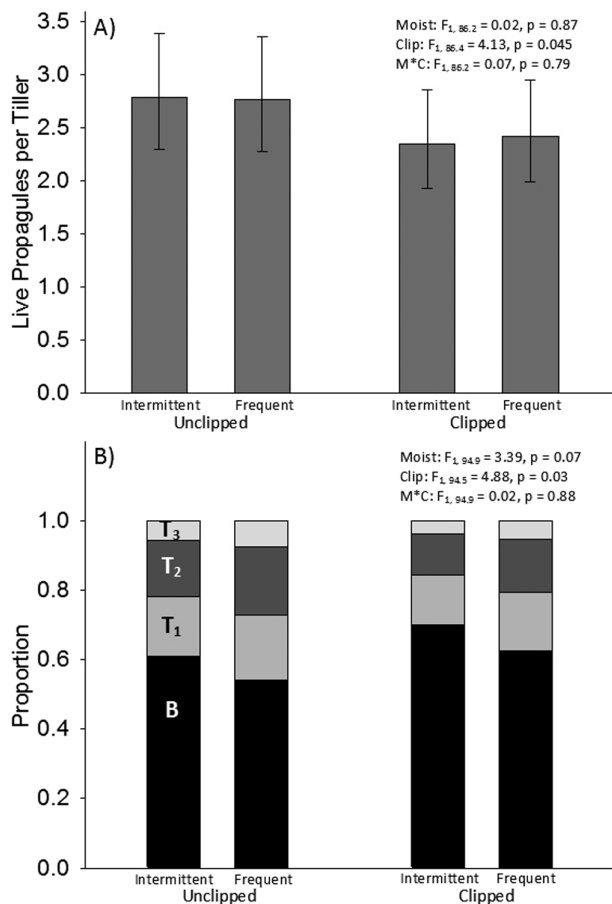


**Figure 3:** (A) live propagules per tiller and (B) propagule development response to temperature and clipping for *P. smithii* under frequent watering conditions. The proportion of live propagules were classified into four developmental/size classes including buds (B), small juvenile tillers/rhizomes ( $T_1$ ), large juvenile tillers/rhizomes ( $T_2$ ) and adult tillers ( $T_3$ ; see Table 1 for more detailed descriptions). Error bars  $\pm 1$  SE based on the statistical model.

unclipped treatments, propagule development did not significantly differ between 12 and 18°C (contrast 12°C versus 18°C:  $t_{184.4} = 0.34, P = 0.74$ ) but did significantly differ between the highest temperature (24°C) and the lower two temperatures (contrast average 12°C and 18°C versus 24°C:  $t_{228.9} = 2.38, P = 0.018$ ). At the lower two temperatures, the odds of a propagule being at a higher developmental stage were 1.48× greater than at the highest temperature.

### Experiment 4: watering frequency and clipping for *P. smithii*

Intermittent watering did not alter the number of live propagules per *P. smithii* tiller at the lowest temperature (Fig. 4A). Similar to Experiment 3, clipping significantly reduced the number of live propagules per tiller as well as propagule development (Fig. 4). Propagule development was marginally reduced by intermittent watering (Fig. 4B). The odds of a propagule being at a higher developmental stage were 1.37× greater for the frequently watered treatment as compared



**Figure 4:** (A) live propagules per tiller and (B) propagule development response to watering frequency and clipping treatments for *P. smithii* at 12°C. The proportion of live propagules were classified into four developmental/size classes including buds (B), small juvenile tillers/rhizomes (T<sub>1</sub>), large juvenile tillers/rhizomes (T<sub>2</sub>) and adult tillers (T<sub>3</sub>; see Table 1 for more detailed descriptions). Error bars  $\pm$  1 SE based on the statistical model.

to the intermittently watered treatment (OR 95% CI [0.98, 1.92]).

## DISCUSSION

As hypothesized, bud availability and outgrowth of the invasive *B. inermis* was greater than the native *P. smithii* across all temperatures and during intermittent watering. Neither species experienced notable amounts of bud mortality when subjected to the different levels of temperature and watering frequency. Axillary bud production is closely tied to tiller growth. As a tiller produces a leaf, it typically produces a bud (Briske 1991; Etter 1951). At the time of plant harvest from the field, buds had already formed at the base of the tillers. Because tillers were fully developed at the start of the experiment, new buds were not produced during the experiment. Therefore, *B. inermis* produced more buds per tiller in the field than *P. smithii* and other dominant grasses

in northern mixed-grass prairie (e.g. Ott and Hartnett 2015a, 2015b; Russell *et al.* 2015). *Bromus inermis*, similar to *P. smithii*, also has an extensive rhizome system from which additional tillers can be recruited from axillary rhizome buds (Ott and Hartnett 2015b; Otfinowski *et al.* 2007). Although multiple axillary buds may be present at the base of a parent tiller, bud dormancy can inhibit the number of buds that are able to be activated and emerge as daughter tillers (i.e. bud outgrowth). Although some buds of both species remained dormant, *B. inermis* initiated a larger percentage of them than *P. smithii*. Therefore, *B. inermis* had a larger replacement ratio of new tillers per parent tiller than *P. smithii*. The greater bud availability per tiller and bud outgrowth of *B. inermis* under the tested range of temperatures and watering frequencies may be a key mechanism facilitating the expansion of *B. inermis* into North American grasslands dominated by *P. smithii*. However, further work is needed to examine this advantage under a broader range of environmental conditions with special consideration of drier and warmer scenarios as well as long-term tiller survival.

Although both *B. inermis* and *P. smithii* are C<sub>3</sub> grasses, warmer spring temperatures did not induce bud mortality. However, the propagule development of *P. smithii* but not *B. inermis* was marginally reduced at 24°C. Optimal physiological conditions for C<sub>3</sub> plant growth decline above 22–30°C at the current global CO<sub>2</sub> concentrations (Ehleringer *et al.* 1997). These optimal conditions may decline at a higher temperature for *B. inermis* than *P. smithii*. Further study may indicate that spring temperatures above 24°C could provide *B. inermis* a competitive advantage over *P. smithii* because tiller recruitment of *P. smithii* would be suppressed at higher spring temperatures. Average spring temperatures may not reach 24°C in the northern Great Plains for another century (Kunkel *et al.* 2013), but 2-week periods of warm spring temperatures that delay *P. smithii* bud development could occur in the next several decades. However, under current spring temperatures, bud and initial tiller development of *B. inermis* and *P. smithii* remain unaffected. This response of both species to short-term warming could differ from their response when their plants are exposed to longer periods of warming (Morgan *et al.* 1994). Long-term conditioning of plants to warming spring temperatures can reduce their photosynthetic capacity (Morgan *et al.* 1994; Williams 1974), which could also reduce bud outgrowth because of reduced carbon accumulation.

In this experiment, short-term reductions in soil moisture did not inhibit propagule development or cause bud death. Similarly, drought did not affect short-term responses such as biomass allocation of *B. inermis* and *P. smithii* seedlings (Dong *et al.* 2012). Leaf production of two invasive species, *B. inermis* and *Agropyron desertorum* (Fisch. ex Link) Schult., was also more tolerant of gradual and rapid water stress over 4 weeks than two native species, *N. viridula* and *Agropyron dasystachyum* (Hook.) Scribn. & J.G. Sm. (Reekie and Redmann 1991). Tiller recruitment (i.e. bud outgrowth) may not be impacted unless drought severity and duration are greater. Longer-term

droughts decreased *B. inermis* shoot dry matter (Donkor et al. 2002), increased *P. smithii* sequestration of non-structural carbohydrates belowground (Morgan et al. 1998) and strongly reduced *P. smithii* annual tiller recruitment (Eneboe et al. 2002). Even under dry conditions, *B. inermis* retained its competitive advantage over *P. smithii* (Nernberg and Dale 1997). Long-term drought likely impacts both tiller recruitment and survival. Precipitation in northern mixed-grass prairie is expected to increase and exhibit greater variability (Kunkel et al. 2013). If the projected increase in precipitation variability in the northern mixed-grass prairie only creates short-term droughts that do not affect bud outgrowth, both *B. inermis* and *P. smithii* will benefit from the projected increase in precipitation. Their increased bud and tiller production will increase bud density and could have a positive legacy effect on ANPP (Reichmann et al. 2013).

At all temperatures, simulated grazing increased bud mortality and reduced bud development of *P. smithii*. The negative effect of clipping on bud outgrowth could be the short-term result of the parent tiller's inability to regrow due to the removal of its apical meristem and the longer response time it takes for daughter tillers to be initiated from dormant buds (Briske 1991; Hyder 1972). Over longer periods of time, grazing usually increases tillering of *P. smithii*. Grazed *P. smithii* tillers produced ~30% more daughter tillers than ungrazed tillers by the end of the growing season (Eneboe et al. 2002). Within 10 days of clipping, tiller production was already similar between unclipped, moderately clipped and heavily clipped *P. smithii* plants (Painter and Detling 1981). As a rhizomatous grass with widely spaced tillers, *P. smithii* may also maintain traits beneficial for grazing avoidance rather than grazing tolerance. For example, *P. smithii* did not alter its short-term carbon allocation pattern following defoliation indicating a lack of grazing tolerance (Painter and Detling 1981). Grazing tolerance is often characterized by rapid bud outgrowth. Despite having similar carbon, nutrient and meristem availability, grazing tolerant *A. desertorum* had greater daughter tiller production than grazing sensitive *Pseudoroegneria spicata* (Pursh) Á. Löve (Caldwell et al. 1981; Mueller and Richards 1986). Certain wheatgrass species, such as *P. smithii* and *P. spicata*, may have greater requirements to break bud dormancy than wheatgrass species such as *A. desertorum* that demonstrate grazing tolerance with rapid bud outgrowth.

## CONCLUSION

Based on bud outgrowth responses, increasing temperatures and precipitation variability will not alter the current invasion of *B. inermis* in the northern mixed-grass prairie of North America. Invasion of *B. inermis* into native *P. smithii* rangeland may also be facilitated by intensive grazing in which *P. smithii* is given inadequate time between grazing events to recover from high bud mortality and lowered bud development of grazed tillers. Bud and tiller production are dependent on one another. Bud outgrowth (i.e. tiller recruitment) produces new tillers and established tillers produce new buds.

Increased temperatures, decreased soil moisture and grazing could alter either of these processes. Future research should examine both bud outgrowth and tiller establishment of more dominant grasses under a wider range of environmental conditions. This current and future bud bank research will provide a mechanistic understanding of our grassland response to climate change in the context of current grazing practices and invasion by non-native perennial grasses.

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