BELOWGROUND BUD BANK DYNAMICS OF GRASSES FOLLOWING EXTREME FIRE AND DROUGHT

A Thesis

by

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ABSTRACT

With the removal of fire and increased grazing pressures in savanna ecosystems worldwide, graminoid abundances have decreased while invasive woody shrubs densities have increased. This has contributed to numerous deleterious ecological and economic consequences for vast semiarid savanna landscapes. Despite tremendous resilience in the majority of resprouting woody plants, previous studies have shown that the use of high-intensity fires during drought can cause woody shrub mortality. However, it is not well-known whether high-intensity fires conducted in during drought are equally deleterious for native grasses. The objectives of this study were to assess the persistence and recovery of native grasses following varying fire intensities to identify how fire intensity impacts bud bank dynamics and evaluate the production of water-repellent soil layers potentially induced by different fire treatments. We established our study in a semiarid Texas savanna rangeland and our treatments consisted of 1) control, 2) low-intensity burn, and 3) high-intensity burn plots. Tillers were collected from two abundant, physiologically-distinct grass species, *Nassella leucotricha* and *Hilaria belangeri,* over the course of a year, and the associated belowground buds were counted and their activity classified as either active, dormant, or dead. Soil cores were taken to assess soil water repellency before and after burning. We found that our treatments did not produce a hydrophobic soil layer. Dead buds significantly increased and bud activity decreased in the high-intensity fire treatment within 24hr post-treatment for both species. Similarly, low-intensity treatment also resulted in an immediate decrease in bud activity for both species, and an increase of dead buds for *H. belangeri*. Despite bud death, *N. leucotricha* individuals resprouted in the high-intensity treatment but not all of *H. belangeri* individuals did. Over 8 months, we found that *N. leucotricha* bud numbers and activity in the high-intensity treatment were equal to the control by the end of our study. *H. belangeri* bud numbers and activity in the high-intensity treatment were significantly lower than the control by the end of the study. Understanding how fire intensity impacts the bud banks of grasses will allow better predictions of grass response to various management tools, such as high-intensity fires.

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CHAPTER I

INTRODUCTION

Native grasses of the Great Plains have adapted to withstand frequent disturbances such as fire, drought, and grazing by resprouting from a belowground bud bank (Wright and Bailey 1982; Benson and Hartnett 2006). These bud banks are a collection of meristematic tissues that can produce new tillers following a disturbance or a sudden increase in resource availability (Dalgleish and Hartnett 2006). Therefore, in the presence of frequent disturbances, especially fire, regrowth from a bud bank that is insulated and protected by a layer of soil offers a competitive advantage (Dalgleish and Hartnett 2009; Russell et al. 2015).

Frequent fires and increased grazing pressures lead to native, perennial graminoid dominance in semi-arid savannas for a multitude of reasons. Most of these ecosystems have developed under fire and grazing pressures and, as such, have exerted selective pressures on the plant community (Milchunas and Laurenroth 1993). As a result, these ecosystems are dominated by growth forms that allocate significant resources belowground, often in a bud bank, exploit brief windows of moisture availability, and regrow quickly and efficiently following defoliation events (Milchunas and Laurenroth 1993; Augustine et al. 2017). In the absence of frequent disturbances, these growth forms are no longer advantageous.

Without frequent fires, dead plant material accumulates and decreases total rates of photosynthesis in native grasses by delaying shoot emergence, increasing the length of time it takes for a shoot to emerge above standing litter, and reducing the rate of tillering which leads to lower tiller densities overall (Knapp and Seastedt 1986). Fires also control native, woody shrubs and annual grasses by directly causing seed and seedling mortality (Vermeire and Rinella 2009; Bragg and Hulbert 1976). Therefore, without fires, brush abundance increases substantially due to increased survival of seedlings (Bragg and Hulbert 1976).

Considering all these points, without the presence of fire in this ecosystem, native grass abundance decreases and brush dominance increases (Bond, Woodward, and Midgley 2005). Brush encroachment leads to a decrease in forage production and, therefore, has a negative effect on livestock production (Ortmann et al. 1998). Brush encroachment also shifts the plant community dynamic toward a brush dominated state which can have a large impact on wildlife habitat and species diversity (Arno 1996; Smit and Prins 2015). This is a problem for rangeland managers because invasive brush is difficult and expensive to remove even when prescribed fire is reintroduced to the system (Trollope and Tainton 1984).

Previous studies have shown that the use of high-intensity fires during times of low water-availability can cause woody shrub mortality (Twidwell et al. 2016). Since most studies have been conducted with moderate fire intensities, graminoid and species-specific responses to high-intensity fires is relatively unknown. Therefore, it is imperative to understand how fire intensity and water availability interact with the bud bank if these high-intensity fires are to be used as a management tool.

Thesis Structure

This thesis is divided into four parts. Chapter 1 consists of an introduction including a literature review, objectives and hypotheses, study area, and literature cited. Chapters 2 and 3 were written as manuscripts to be submitted to peer reviewed journals. Specifically, Chapter 2 will be submitted to the *Journal of Applied Ecology* and Chapter 3 will be submitted to the *American Journal of Botany*. Chapter 4 is an overall summary of the purpose, results, and importance of this thesis project.

Literature Review

Fire

Fire influences community composition and processes by altering soil temperature, modifying soil moisture, changing soil nutrients, influencing the amount of light that reaches the ground, stimulating microbial activity, and removing senesced plant material (Willems 1983; Vermeire and Rinella 2009; Turner et al. 1997). In semi-arid savannas, decomposition rates of above-ground plant litter tend to be low due to poor litter quality (low N content) and decreased water availability (Zhang et al. 2008). Therefore, fires release nutrients that were previously immobilized in accumulated litter (DeBano et al. 1998). The increased rates of nutrient turnover due to frequent fire is essential for maintaining high primary productivity in grasslands and savannas (Woodmansee and Wallach 1981).

Burning can also stimulate grasses depending upon the season of the burn and, therefore, their state of activity due to differences in phenology (Ewing and Engle 1988).

C_3 (cool season) species are dormant during the hot summer months so fires during these times increase C_3 functional group dominance (Russell et. al. 2015). For the same reason, winter and spring burns stimulate C_4 (warm season) species (Steuter 1987, Russell et. al. 2015). Overall, season of fire directly manipulates bud bank size by altering bud mortality, activity, and dormancy (Steuter 1987, Russell et al. 2015).

Bud bank dynamics may also be influenced by functional group, specifically C_3 vs C_4 phenology. There is evidence that C_4 grass species maintain larger bud banks in which the buds live multiple years (Ott and Hartnett 2012; Benson, Hartnett, and Mann 2004). This results in tiller recruitment coming from multiple previous years' bud cohorts (Ott and Hartnett 2012; Benson, Hartnett, and Mann 2004). In contrast, C_3 grass species maintain smaller bud banks, with buds usually surviving for a single year (Ott and Hartnett 2012; Benson, Hartnett, and Mann 2004). Given these differences, buds seem to play different roles in the growth dynamics of each functional group (Ott and Hartnett 2012).

Fire frequency can also determine bud bank size by altering competition dynamics between grasses and forbs. When fires are completely excluded from a system, native, perennial grass bud banks decrease while the bud banks of forbs increase (Benson et al. 2004). Similarly, bud bank size increases following annual burning in tallgrass prairie (Benson, Hartnett, Mann 2004; Dalgleish and Hartnett 2009). Grass species with large belowground bud banks are able to opportunistically exploit periods of increased resource availability, such as following precipitation or fire (Dalgleish & Hartnett, 2006). In juxtaposition, when grasses are meristem limited, they are unable to respond and take advantage of pulses in resource availability (Dalgleish & Hartnett, 2006). Therefore, bud bank size is important because it directly relates to the ability of a plant to utilize its reserves and respond to disturbances and pulses of high resource availability (Russell et al 2015).

Fires also control undesirable species such as invasive resprouting woody brush and annual grasses in semi-arid savanna systems. For example, woody species such as *Juniperus asheii* (Ashe juniper) are typically vulnerable to fire-induced mortality (Ortmann et al. 1998). Annual grasses, being obligate seeders, see reductions in numbers through direct seed mortality due to fire (Vermeire and Rinella 2009). When fire is removed, these species increase in abundance and encroach (Peterson, Reich, and Wrage 2007; Heisler, Briggs, and Knapp 2003).

Morphological adaptations of graminoids can also play a role in fire response. Rhizomatous grasses are typically resistant to moderate intensity fires due to their axillary buds being deeply buried beneath the soil surface (Wright and Bailey 1982; Dalgleish and Hartnett 2009; Clarke et al. 2013). Bunchgrasses grow from the center of the plant outward; this method of growth promotes litter accumulation at the center of the grass (Wright 1971). Increasing the fuel load in and around the crown results in greater heat duration and heat dosage at the center of the plant (Wright 1971; Wright and Bailey 1982). Because of this, bud mortality in bunchgrasses has been reported to be greater at the center of the plant as opposed to the edges where there is less accumulation of old growth (Morgan and Lunt 1999). Wright (1971) found that squirrel-tail (*Hordeum jubatum*) is more tolerant to burning than needle-and-thread (*Hesperostipa comata*) due to the low density of dead plant material that accumulates in and around the crown which leads to very minimal heat penetration. Small bunchgrasses, which contain only a few current-year tillers, will usually survive a fast-moving fire because of the lack of accumulation of old growth (Wright and Bailey 1982). Overall, retention of senescent biomass and plant compactness lead to increased residence time of fire (Gonzalez, Ghermandi, and Pelaez 2015).

The depth at which the bud bank sits below the soil surface may also contribute to bud activation and death. Soil is considered a good insulator, and, therefore, retards the downward conduction of heat into the soil (Valettel et al. 1994; Clarke et al. 2013). Considering this, a stoloniferous and a caespitose grass are likely to have their bud banks at different depths beneath the soil surface due to their differences in vegetative growth. Therefore, growth forms may be integral to understanding differential mortality rates between grass species.

Drought

The meristem limitation hypothesis states that bud banks increase along a precipitation and productivity gradient (Dalgleish and Harnett 2006). When water availability is low, plants restrict investment of new tissue to belowground buds due to low photosynthesis rates which results in smaller bud banks, limited bud production, and limited bud maintenance (Dalgleish and Harnett 2006). Smaller bud banks equate to a decreased potential to produce tillers. Bud demography is also tightly linked to rhizome production and senescence and, therefore, affects the compactness of grasses (Dalgleish and Harnett 2006; Benson, Hartnett, and Mann 2004).

Drought not only reduces belowground bud bank density but also aboveground stem density (Carter et al., 2012). Despite these changes to plant structure, they found that bud density recovered quickly and even that bud production was higher following the year after drought. Therefore, although drought reduces bud bank and stem density, there is a possibility for grasses to recover.

Water stress also changes community structure. Stressors such as drought in combination with fire can lead to depletion of buds and reserves that may inhibit resprouting following fire (Dalgleish and Harnett 2006). Grasslands in arid regions usually support perennial grasses with small bud banks, and, in part because of this, these regions usually show a higher dominance of annual grasses (Qian et al. 2017). Due to short-term drought, VanderWeide and Hartnett (2015) observed a decline in overall herbaceous species richness both above and below the soil surface.

Soil Properties

Soil is considered an effective insulator and retards the downward conduction of heat (Valettel et al. 1994). Choczynska and Johnson (2009) found that, even under extreme fire conditions, lethal temperatures for buds did not occur below 2 cm in depth. Valletell et al. (1994) found a similar trend in which, even with higher intensity fires, there was a significant decrease in temperature as soil depth increased. Soil moisture can alter this trend because water increases the specific heat of the soil and decreases the thermal conductivity. Despite many studies on the interaction of soil moisture and fire, Choczynska and Johnson (2009) found that the most important factor influencing soil heating was the surface temperature curve (fire residence time and maximum surface temperature) rather than whether the soil was wet or dry.

The temperature levels recorded in high-intensity fires may produce a water repellent layer in the soil which could increase erosion, delay vegetation regrowth, and decrease infiltration (DeBano and Rice 1973, DeBano and Krammes 1966, Certini 2005). The depth and thickness of the water-repellent layer that develops after a fire depends on the intensity of the fire and the amount and type of vegetation and litter present (DeBano and Rice 1973). The type of vegetation is important because certain species, such as Chaparral brush species, create more hydrophobic organic substances than others (DeBano and Rice 1973, DeBano and Krammes 1966). In high-intensity fires, the water-repellent layer often develops at the surface or just below the soil surface (DeBano and Rice 1973). In general, water repellency usually develops within 10-15 cm of the soil surface (DeBano and Rice 1973). DeBano and Rice (1973) found that the effect of a water repellent soil layer on vegetation and hydrology is reduced greatly following the first year but may continue to have an effect up to 5 to 10 years after the fire. Therefore, understanding the factors influencing the production of a water repellent layer in the soil following high-intensity fires is imperative to evaluating the survivability of native grasses.

Study Area

The study site is located at the Sonora Texas A&M Agrilife Research Station (SARS) which is situated on the western edge of the Edwards Plateau ecoregion (-100.574°, 30.251°). SARS is situated in a semi-arid, savanna system that experiences a bimodal precipitation pattern. The average annual precipitation varies from 356 to 889 mm, with the majority falling in the spring and fall. The average annual temperature ranges from 14 to 21 ˚C, with summer temperatures reaching up to 38 ˚C. The soils tend to be very shallow, and exposed limestone bedrock is a common sight. Our site is situated in a savanna system; therefore, the dominant vegetation consists of a mosaic of trees and graminoids. The dominant trees in the area are *Quercus spp.* and *Juniperus spp.*. The dominant graminoid species are *Hilaria belangeri* (Steud.) Nash, *Aristida spp*., *Bouteloua curtipendula* (Michx.) Torr., *Nassella leucotricha* (Trin. & Rupr.) R.W. Phol, and *Pleuraphis mutica* Buckley. The western Edwards Plateau experiences a fire return interval of 1-12 years (Stambaugh et al. 2014). Historically, fires were more common during late winter and late summer when grasses were usually dormant or dry and lightning strike frequency was high (Stambaugh et al. 2014).

For our study, we focused on two of the dominant graminoid species in our plots: *N. leucotricha* and *H. belangeri*. Out of the other dominant grasses in the Edwards Plateau ecoregion, these two were the most abundant in our plots. *N.* is a C_3, caespitose grass while *H. belangeri* is a C_4, stoloniferous grass.

Objectives and Hypotheses

Native grasses of the Great Plains depend on their bud banks to persist following fire and to take full advantage of post-fire conditions. Therefore, it is critical to understand how fire intensity and water stress interact with bud bank dynamics. This is especially true if high-intensity fires during times of drought are to be used as a management tool to control/reduce mature woody brush densities.

Therefore, the objectives of this study are to 1a) identify the effect of fire intensity on soil water repellency, 1b) assess immediate, within 24hr of treatment, bud response to fire intensity, 1c) assess how phenological and morphological characteristics of grasses impact their response to the treatments, 2a) assess the persistence of native grasses following extreme fire and drought, and 2b) assess how bud dynamics differ between functional groups over a single growing season.

With these objectives in mind, we can ask a series of questions: What are the immediate responses graminoid bud banks have to fire? And how may growth form and phenology factor into these responses? To address these questions, the following hypotheses will be tested:

Hypothesis 1a [H1a]—Fire intensity will have no effect on soil hydrophobicity. Despite some concerns indicating potential effects of extreme fire on soil hydrophobicity, water repellent layers are not expected to form in our plots. Previous studies that have shown the production of water repellent layers in response to fire have been in ecosystems in which the dominant vegetation has been shrubs or trees that contain hydrophobic waxes and oils.

Hypothesis 1b [H1b]—Low-intensity fire should lead to a high number of active buds in *H. belangeri* due to its summer activity and less so for *N. leucotricha* due to summer dormancy. Native perennial grasses in the Edwards Plateau are adapted to local conditions that usually include drought during the late summer months and low-intensity fires during these times. As such, grasses subjected to water stress and low-intensity fires are expected to have high survival rates and lead to high bud activation.

Hypothesis 1c [H1c]—Greater bud bank depth will result in lower bud mortality. Soil is a good insulator and, although high-intensity fires may lead to greater heat movement down into the soil than low-intensity fires, the location of underground buds may lead to better protection, especially if the aboveground growth is not compact. Due to growth form, caespitose grasses should have deeper buds than stoloniferous grasses. Therefore, we should see high mortality in *H. belangeri*, especially in the high-intensity treatment, because stolons and their associated buds are not protected by the soil.

Some other questions we can address are: How quickly will it take for bud banks to recover? What are the patterns of bud dormancy, death, and activation over the year? What impact does growth form and photosynthetic pathway have on these patterns?

Hypothesis 2a [H2a]—Regardless of fire intensity, we should see both *N. leucotricha* and *H. belangeri* recover. Full recovery may not be evident until the second growing season (Russell et al. 2015) but we should see steady recovery in the first growing season. The combination of low water stress, removal of competitors, and increase in nutrient availability following our treatments should result in quick recovery following removal of aboveground growth and bud death regardless of fire intensity.

Hypothesis 2b [H2b]—We expect to see different bud activity, dormancy, and total bud peaks between *N. leucotricha* and *H. belangeri.* These grasses are active during different times of the year. *N. leucotricha*, being a C_3 grass, should be active during the cooler parts of the year and *H. belangeri*, being a C_4 grass, should be active during the hotter months (Ewing and Engle 1988). *H. belangeri* should have a larger bud bank, overall, and a larger amount of dormant buds than *N. leucotricha*. In many cases, C_4 grasses maintain larger bud banks than C_3 grasses, and their buds live multiple years (Benson, Hartnett, and Mann 2004). Therefore, understanding how phenology differences due to functional group, as well as fire intensity and timing, affect bud bank dynamic will be important management concerns.

Implications

Most perennial grasses primarily reproduce clonally via axillary buds associated with a bud bank. In fact, Benson and Hartnett (2006) found that over 99% of all established shoots at the end of the growing season were clonally produced as opposed to establishment via seed. This establishes the importance of belowground bud bank dynamics in annual regeneration. Knowing this, this study serves to increase our knowledge on how bud banks of grasses in a semi-arid savanna system respond to high-intensity fires and the implication of these dynamics on community composition.

This is especially relevant when using high-intensity fires during times of drought as a management tool to remove invasive shrubs from the landscape (Twidwell et al. 2016). Will intervening with fire simply degrade the ecosystem by overcoming the resilience of the herbaceous community? Or will the energy input transition the community back to a savanna system? This study will hopefully help answer these questions and indicate whether these treatments should be used as a management tool in encroached semi-arid savanna systems.

There is evidence that the bud bank dynamics of C_3 and C_4 grasses are not necessarily congruous. In many cases, C_4 grasses maintain larger bud banks than C_3 grasses, and their buds live multiple years (Benson, Hartnett, and Mann 2004). These grasses are also active during different times of the year, with C_3 grasses being active during the cooler season and C_4 grasses being active during the hotter months (Ewing and Engle 1988). Therefore, understanding how phenology and life-history traits affect bud bank dynamics and how fire intensity and timing affect these dynamics will be important in management situations.

Vegetative growth comes in many different forms and not all growth forms may react similarly to the same stimuli. The bud banks of caespitose grasses are insulated by the soil. On the other hand, stoloniferous grasses will experience higher temperatures due to lack of insulation. However, caespitose grasses, especially older individuals, may experience greater temperatures than stoloniferous grass due to detritus accumulation around the crown. As such, studying the different growth forms of these grasses is important for management concerns.

Overall, the results from this study can be used as a predictive tool for herbaceous plant responses to management practices using extreme burns for the removal of woody plants. These results will also provide insight on species-specific bud bank dynamics which is important due to the general lack of species-specific data. Data on the effect of fire intensity on the graminoid bud bank will allow for better management actions in savanna and grassland systems.

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CHAPTER II

IMMEDIATE BELOWGROUND BUD BANK RESPONSES TO FIRE INTENSITY IN A SEMI-ARID SAVANNA SYSTEM

Chapter Summary

In systems dominated by perennial grasses, belowground bud banks determine plant community response to disturbances such as fire. As such, the use of high-intensity fires as a possible management tool will have implications for plant community dynamics in savanna and grassland systems. This study examined the immediate (<24hr) bud bank dynamics of a C_3 caespitose grass, *Nassella leucotricha*, and a C_4 stoloniferous grass, *Hilaria belangeri*, and investigated potential soil hydrophobicity following fires of varying intensity in a semi-arid savanna system in the Edwards plateau ecoregion of Texas. Treatments included high-intensity, low-intensity, and no burn (control) treatments. Belowground axillary buds were counted and their activities classified to determine immediate effects of fire intensity on bud bank activity, dormancy, and mortality. High-intensity burns resulted in immediate mortality of *N. leucotricha* and *H. belangeri* buds while low-intensity burns resulted in immediate mortality of only *H. belangeri* buds ($P < 0.05$). Active buds decreased following high-intensity and low-intensity burns for both species ($P < 0.05$). In contrast, bud activity, dormancy, and mortality remained constant in the control. Of the 48 individuals monitored in the high-intensity treatment, half of which were *N. leucotricha* individuals and half *H. belangeri* individuals, 100% of *N. leucotricha* individuals resprouted while only 25% of *H.*

belangeri resprouted ($P < 0.0001$) 3 weeks following treatment application. Bud depths were significantly different between the two species and may account for these differences, with average bud depths for *N. leucotricha* being 1.3 cm deeper than *H. belangeri* ($P < 0.0001$). We also found no incidence of soil water repellency following fire treatments. Therefore, fire intensity directly manipulates bud activity, dormancy, and mortality for these two species.

Introduction

In ecosystems dominated by perennial grasses, aboveground growth and persistence following disturbances is often determined by regrowth from a belowground bud bank (Rogers & Hartnett, 2001; Benson & Hartnett, 2006; Dalgleish & Hartnett, 2009). This type of growth is overwhelmingly prolific, with estimates of more than 99% of all new tiller growth originating from belowground buds (Benson & Hartnett, 2006). Therefore, bud bank size determines the growth potential of perennial grasses but also directly determines a plant's ability to activate reserves, respond to disturbances, and react to pulses of high resource availability (Russell et al., 2015). Given this, bud bank size is a key component in a plant community's response to disturbances.

Semi-arid savanna systems have developed under frequent fires and grazing pressures, and, as such, have exerted selective pressures on plant community structure and composition (Milchunas & Laurenroth, 1993). In the presence of these disturbances, especially fire, regrowth from a bud bank that is insulated and protected by a layer of soil offers a competitive advantage to herbaceous species that vegetatively regenerate

(Rogers & Hartnett, 2001; Dalgleish & Hartnett, 2009; Russell et al., 2015). With the suppression of natural processes, there has been an increase in woody shrub invasions into grass dominated communities.

Prescribed fires have been known to sustain grass dominance. However, once invasion by woody species proceeds beyond a certain threshold, reintroducing fire into the system is seldom a viable means to return to a grass-dominated state. Nevertheless, there has been some research showing that the use of high-intensity fires during times of drought can result in mature woody shrub mortality (Twidwell et al., 2016). However, there has been little research into how these management efforts may affect grasses. Most studies to date have been conducted with moderate intensity fires, so quantitative assessments of graminoid response to high-intensity fires are relatively unknown. Given the potential desirability to use these conditions as a management tool to remove woody shrubs and increase grass cover, it is important to understand how fire intensity affects with bud bank of herbaceous plant species.

The temperatures reached in high-intensity fires may produce a water repellent layer in the soil (DeBano & Rice, 1973; DeBano & Krammes, 1966; Certini, 2005). The creation of this layer can be particularly damaging because it has the propensity to increase erosion, delay vegetation regrowth, and decrease infiltration rates (DeBano & Rice, 1973; DeBano & Krammes, 1966; Certini, 2005). These effects normally decrease after the first year but may continue up to 5 to 10 years following the event (DeBano & Rice, 1973). The development of a water-repellent layer likely depends not only on fire temperatures, but also by soil properties and the type and amount of vegetation and litter present (DeBano & Rice, 1973). Vegetation type is important to note because certain species, such as Chaparral brush species, create more hydrophobic organic substances than others (DeBano & Rice, 1973; DeBano & Krammes, 1966). Due to the potential negative biogeochemical consequences, it is imperative to determine if a hydrophobic soil layer can form following high-intensity fires in an encroached semi-arid savanna system in order to evaluate the survival of native grasses.

The depth at which the bud bank sits below the soil surface contributes to grass survival following disturbances, especially in the case of fires. Soil is an insulator and retards the downward movement of heat into the soil (Valettel et al., 1994; Clarke et al., 2013). As such, bud position in relation to the soil surface is important to note and most likely different for different grass growth forms. Stoloniferous and caespitose grasses typically have different bud depths due to their different vegetative growth strategies. Therefore, growth form traits and life-history strategies may be integral to understanding the effect of fire intensity on the bud bank.

Season of burn differentially stimulates grass species due to temporal variation in dormancy and growth determined by functional group (Ewing & Engle, 1988). C_3, cool season, species are dormant during the hot summer months (Steuter, 1987; Russell et al., 2015). Fires during these times increase C_3 functional group dominance (Steuter, 1987; Russell et al., 2015). For the same reason, winter and spring burns stimulate C_4, warm season, species (Steuter, 1987; Russell et. al., 2015). Consequently, season of fire has a direct impact on bud mortality and bud bank size due to differences in grass phenology (Steuter, 1987; Russell et al., 2015).

This study examined the bud bank dynamics of two, native perennial grass species with contrasting growth forms and photosynthetic pathways in a semi-arid savanna system. The objectives of this study were to 1) identify the effect of fire intensity during drought on soil water repellency, 2) assess the immediate bud responses of a C_3 caespitose grass and a C_4 stoloniferous grass, 3) and assess how functional group and morphological characteristics of grasses influence their response to the treatments. We expected to see bud bank dynamics differ between these species based on differential growth form traits, photosynthetic properties, and bud depth differences.

Materials and Methods

Site description

Research was conducted at the Sonora Texas A&M Agrilife Research Station (SARS), which is situated on the western edge of the Edwards Plateau ecoregion in Texas (-100.574°, 30.251°). This site is a semi-arid, savanna system that experiences a bimodal precipitation pattern. The average annual precipitation varies from 356 to 889 mm, with the majority falling in the spring and fall (Fig. 1). The average annual temperature ranges from 14 to 21 ˚C, with summer temperatures reaching up to 38 ˚C. The western Edwards Plateau experiences a fire return interval of 1-12 years (Stambaugh et al., 2014). Historically, fires were more common during late winter and late summer when grasses were usually dormant or dry and lightning strike frequency was high (Stambaugh et al., 2014).

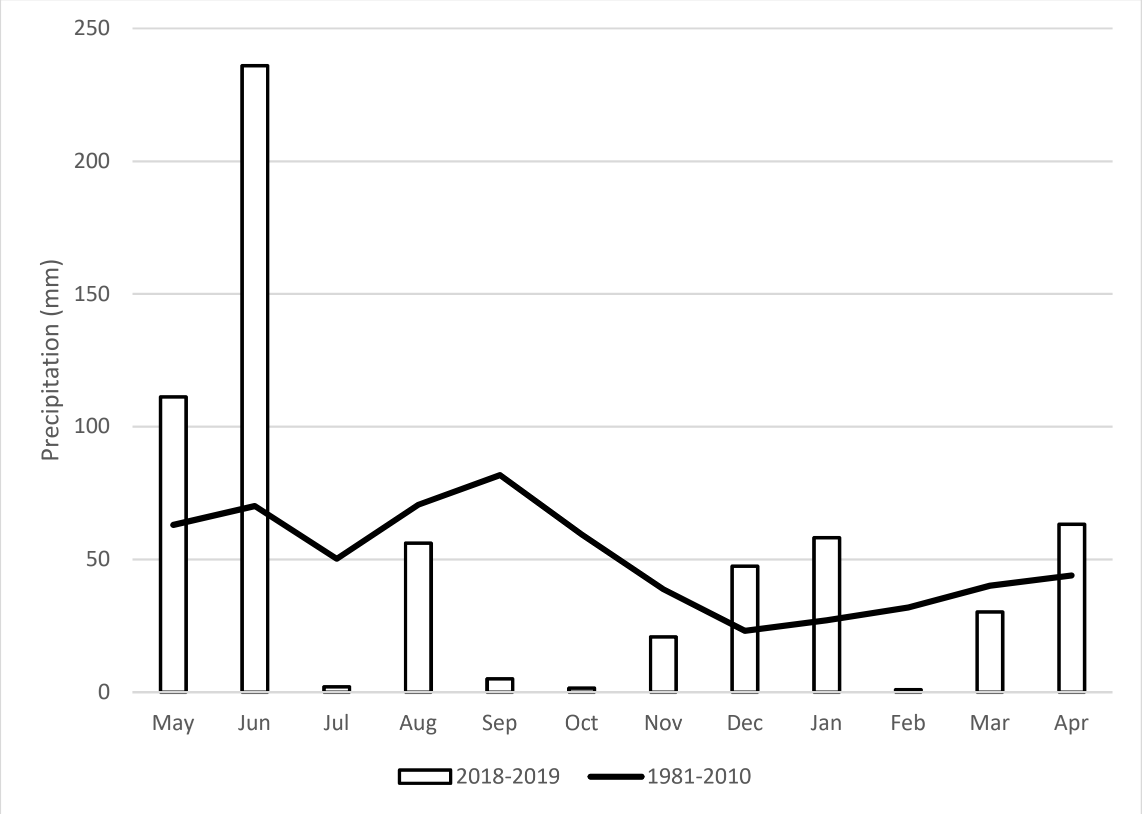

Figure 1: Study site mean precipitation.

The soils are in the Tarrant soil series (Clayey-skeletal, smectitic, thermic Lithic Calciustolls; USDA, 2016). Therefore, they tend to be very shallow, and exposed limestone bedrock is common. The dominant vegetation consists of a mosaic of trees and graminoids. The dominant trees in the area are *Quercus spp.*, *Juniperus spp.*, and *Prosopis glandulosa* Torr.. The dominant graminoid species are *Hilaria belangeri* (Steud.) Nash, *Aristida spp*., *Bouteloua curtipendula* (Michx.) Torr., *Nassella leucotricha* (Trin. & Rupr.) R.W. Phol., and *Pleuraphis mutica* Buckley.

Experimental design and fire measurements

Fire treatments were arranged in a randomized design with three treatments (no burn, low fire intensity, and high fire intensity), replicated 12 times, applied to 36 experimental plots. Each plot was 100 m^2 and centered on a mature (10+ years) mesquite shrub ranging from 3-5m in height. The last time our study site was burned was in August of 2000, in which a high-intensity prescribed burn was conducted. Historically, our site was moderately grazed by sheep and goats. However, one growing season prior to our burns, the pasture containing our study site was rested and all domesticated grazing ceased since.

In early spring 2018, the entire pasture, with exception of our 36 experimental plots, was burned in order to reduce surrounding fuel loads in preparation for our experimental research fires. Each plot had a 5 m fire-break bulldozed on each side. The low-intensity fires required an addition of 61±1 kg of dry hay in order to produce a continuous, low-intensity fireline (Table 1). The high-intensity fires were achieved by adding 201±1 kg of supplemental fuels, specifically by using previously harvested and dried juniper branches along with hay to simulate additions added to the low-intensity treatment. Fuel addition averages and total fuel consumed averages can be seen in Table 1. Each plot was burned using a drip torch by means of a ring fire method. All fire treatment plots were burned between July 30th and August 4th.

During implementation of the experimental fire treatments, weather conditions were monitored along with wind speed and direction adjacent to the plots. Two thermal imaging cameras were used to record fine-scale fire behavior. Fuel bed and fuel consumption were characterized using standard methods outlined by Byram (1959). Fuel consumption, along with the rate of spread, were used to estimate fireline intensity.

Table 1: Fuel additions and consumption at the plot level for low-intensity and high-intensity treatments.

	Mean ± SE	Maximum	Minimum
Hay Addition (kg)	61 ± 1	96	47
Juniper Addition (kg)	201 ± 1	218	197
Fuel consumed (low-intensity; kg)	55 ± 1	80	46
Fuel consumed (high-intensity; kg)	259 ± 3	294	243

Sampling

Two grass species, *N. leucotricha* and *H. belangeri*, were selected for focus in this study due to both their relative abundance at the site and for their contrasting phenological and growth form characteristics (Table 2). Within each large 100m^2 plot, two 1 m^2 mini-plots were demarcated with rebar (Fig. 2). One of these mini-plots was created around a patch of *N. leucotricha*, and the other around a patch of *H. belangeri*. Therefore, each large plot had one *N. leucotricha* mini plot and one *H. belangeri* mini-plot. These mini-plots served as a reference group for tiller collections described in the next section. Within each of these mini-plots, two individuals were permanently marked (Fig. 2). Therefore, four individuals (two per species) were marked for each large plot and were monitored for regrowth three weeks following treatment application.

Tillers were harvested from three randomly determined individuals of each grass species inside each plot (Fig. 2). These tillers were collected from individuals in similar phenological stages as the permanently marked individuals using the classification system of Moore et al. (1991). Plants visibly damaged by herbivores, insects, or pathogens were excluded.

Harvesting tillers consisted of removing 3-6 tillers from individual plants using a trowel to keep both aboveground and belowground structures intact. Only two tillers were used for analysis, but more were harvested in case some tillers were unusable due to disease, herbivory, accidental damage, or other unforeseen complications.

The buds associated with these tillers were counted and their activity classified as either active, dormant, or dead using the Tetrazolium and Evans Blue staining procedures established by Busso (1989). Tillers were submerged in Tetrazolium solution for 24 hr at room temperature. Dormant and dead buds retained a white/yellowish color while active buds were stained pink. Each tiller was then submerged in Evan's Blue solution for 20 min. If buds were dormant, they retained their white pigmentation, while dead buds were stained a dark blue.

The day before the fire treatments were applied, bud bank depth was measured. To do this, two random individuals of each species from each plot (4 individuals total per plot) were selected (Fig. 2). A hole was dug at the base of each individual grass so that the bud bank was exposed. Bud depth was recorded as the distance between the soil surface and the beginning of the root system.

To investigate soil water repellency, three 15-cm soil cores were collected from each plot before and after burning using a 25-mm diameter soil probe (Fig. 2). If there was an ash layer, it was scraped away to expose the soil beneath before taking the core. After collection, cores were stored in a freezer until processed. Each soil core was left to air dry for 48 hr before processing. Following this, each soil core was separated into 3 equal segments (~5 cm each) and sieved with a 0.841 mm mesh (20 mesh). For each segment, a water drop penetration test (WDPT) was conducted as described by Leelamanie et al. (2007). The WDPT consists of placing a single drop of water onto the soil surface and measuring the amount of time until complete penetration occurs.

Samples were put into weighing dishes and leveled. One drop of deionized water (approximately 50 ± 5 μL) was dropped onto the soil surface using a standard medicine dropper. Each drop was released at a height of 10 mm above the sample in order to minimize the cratering effect that may occur on the soil surface.

A stopwatch was used to record the time it takes for the water droplet to completely penetrate the soil surface. Repellency levels (retrieved from Steenhuis et al., 2001) are shown and defined in Table 3.

Table 2: Summary of species morphological and phenological characteristics.

Scientific Name	Common Name	Phenology	Growing Season	Growth Form
Nassella leucotricha	Texas wintergrass	C_3	Spring, Fall	Caespitose/bunchgrass
Hilaria belangeri	Common curly mesquite	C_4	Summer	Stoloniferous

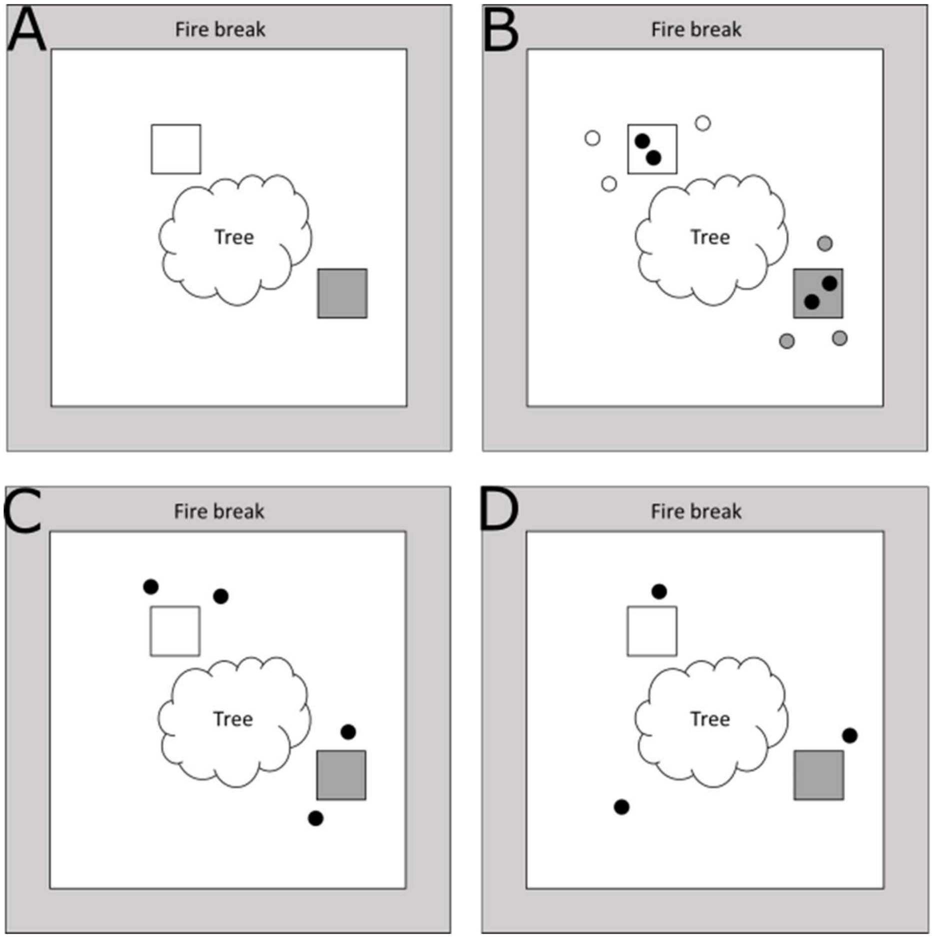

Figure 2: Visual representations of the main methodologies conducted in our experimental plots. All plots were 100 m^2 and centered on a mature mesquite shrub, with a 5 m firebreak around the periphery. A) The two smaller squares are an example of where mini-plots were created; one mini plot per species in each plot. B) The black circles inside the mini-plots represent permanently marked individuals evaluated for regrowth 3 weeks following treatment application; circles outside mini-plots represent individuals chosen for tiller collections and bud assessments. C) Black circles represent individuals chosen for bud bank depth measurements; 2 individuals per species in each plot. D) Black circles represent locations where soil cores were taken 24 hr before and after treatment application; 2 soil cores taken near mini-plots and the last taken randomly from another section of the plot.

Table 3: Definition of repellency classes. Retrieved from Steenhuis et al. (2001).

Class	Time for water to infiltrate (sec)	Description
0	0-5	Not water repellent
1	5-60	Slightly water repellent
2	60-600	Moderately water repellent
3	600-3600	Severely water repellent
4	> 3600	Extremely water repellent

Statistical analyses

Immediate bud response data were analyzed using ANOVAs (MIXED procedure of SAS; Littell et al., 2006). The model included sampling period (pre and post fire), fire intensity, and their interaction as fixed effects. The analyses were completed by species, with total, active, dormant, and dead buds as response variables. Bud depth data was analyzed using a Wilcoxon test to compare *N. leucotricha* to *H. belangeri*. Soil hydrophobicity data were analyzed using an ANOVA with sampling period (pre and post fire), fire intensity, and their interaction as fixed effects. Reemergence of our two grass species following treatments was analyzed using a Fisher's exact test and a Chi-square test. A Fisher's exact test was used for the curly mesquite data because over 20% of expected counts were less than five. Statistical significance was set at $P < 0.05$ for all models.

Results

Nassella leucotricha dynamics

There was a significant difference in total buds between pre- and post-treatment sampling times for both the low- and high-intensity treatments but not for the control

(Table 4). When comparing pre- and post-treatment values, total bud numbers in the high-intensity treatment decreased by 18% (decrease of 0.39±.13 buds tiller^{-1}; P = 0.0021; Fig. 3) following treatment application. Similarly, in the low-intensity fire treatment, total bud numbers decreased by 17% (decrease of 0.37±0.13 buds tiller^{-1}; P = 0.0037; Fig. 3) following treatment application. Overall, total buds decreased following both fire treatments (Fig. 3).

There was a significant effect of fire intensity on the number of active buds (Table 3). When comparing pre- and post-treatment values, active bud numbers in high-intensity fire treatment decreased by 82% (decrease of 0.80±0.13 buds tiller^{-1}; $P <$ 0.0001; Fig. 3) following treatment application. Similarly, in the low-intensity treatment, active bud numbers decreased by 45% (decrease of 0.51±0.13 buds tiller^{-1}; P = 0.0001; Fig. 3) following treatment application. Overall, our fire treatments significantly decreased bud activity while the control treatment was unaffected. Following our fire treatments (post 24hr sampling period), the total number of active buds was significantly different between the low-intensity and high-intensity treatments. The low-intensity treatment, post-treatment, had 0.46±0.16 more buds tiller^{-1} than the high-intensity treatment post-treatment (P = 0.0054).

There was no significant difference in dormant buds between pre- and post-treatment sampling periods for any of our treatments (Table 3).

There was a significant difference in dead buds between pre- and post- treatment sampling periods for the high-intensity treatment but not the low-intensity or control treatments (Table 3). When comparing pre- and post-treatment values, dead bud numbers in the high-intensity treatments increased by 237% (increase of 0.23±0.07 buds tiller^{-1}; $P = 0.0006$; Fig. 3) following treatment application.

Table 4: Immediate (<24hr) fire intensity effects on the number of total, active, dormant, and dead belowground buds belonging to *N. leucotricha* tillers. All bud means are given in buds tiller^{-1}. Means within bud classification are similar when followed by a common letter (P>0.05).

Bud Classification	Pre-treatment			Post-treatment		
	Control	Low	High	Control	Low	High
Total	2.3±0.1a	2.2±0.1a	2.2±0.1a	2.1±0.1a	1.9±0.1b	1.8±0.1b
Active	0.9±0.1ab	1.2±0.1a	1.0±0.1a	1.0±0.1a	0.6±0.1b	0.2±0.1c
Dormant	1.2±0.1a	1±0.1a	1.1±0.1a	1.0±0.1a	1.1±0.1a	1.3±0.1a
Dead	0.2±0.1ab	0.1±0.1a	0.1±0.1a	0.1±0.1a	0.1±0.1a	0.3±0.1b

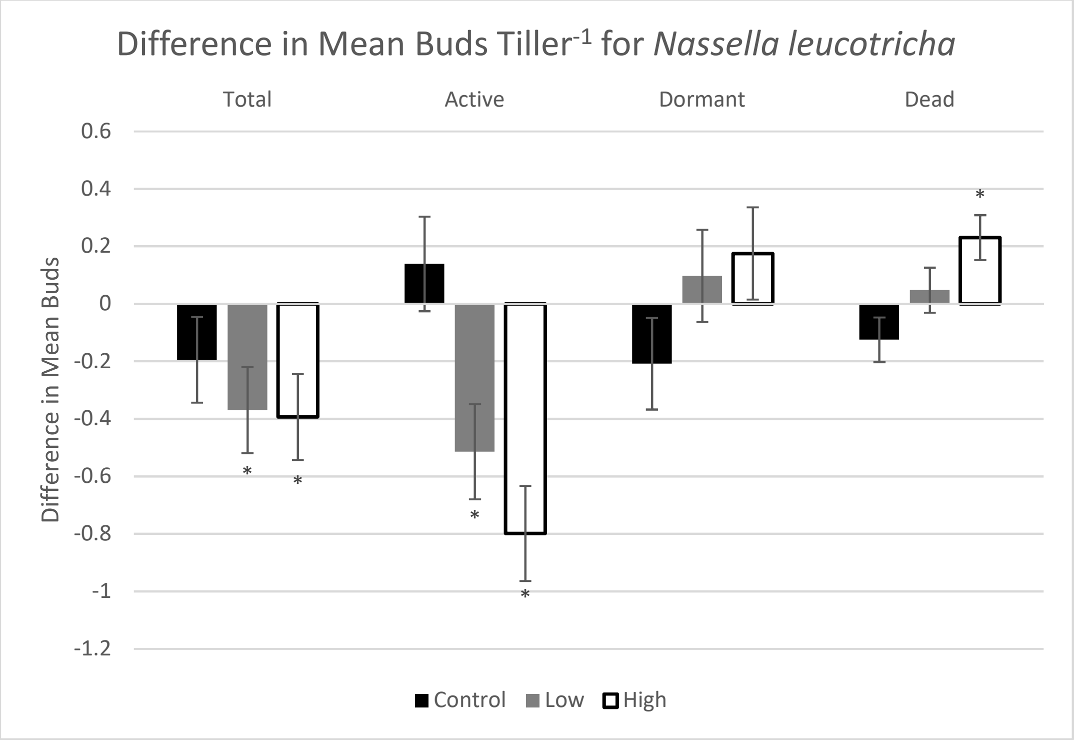

Figure 3: Difference in mean buds tiller^{-1} for *N. leucotricha* between pre- and post-treatment values. Bars with an asterisk (*) indicate significant differences.

Hilaria belangeri dynamics

There was a significant difference in total buds between pre and post treatment sampling times for both the low and high-intensity treatments but not the control (Table 5). When comparing pre- and post-treatment values, total bud numbers in high-decreased by 38% (decrease of 1.25±0.16 buds tiller^{-1}; $P < 0.0001$) following treatment application. Similarly, in the low-intensity treatment, total buds decreased by 34% (decrease of 1.14±0.16 buds tiller^{-1}; $P < 0.0001$) following treatment application. Overall, fire treatments led to a significant decrease in the total number of buds (Fig. 4).

There was a significant difference in active buds between pre and post treatment sampling periods for both the low and high-intensity treatments but not the control (Table 5). When comparing pre- and post-treatment values, active bud numbers in the high-intensity treatment decreased by 89% (decrease of 1.54±0.15 buds tiller^{-1}; $P < 0.0001$; Fig. 4) following treatment application. Similarly, in the low-intensity treatment active buds decreased by 69% (decrease of 1.22±0.15 buds tiller^{-1}; $P < 0.0001$; Fig. 4) following treatment application. Overall, our fire treatments led to a decrease in the number of active buds (Fig. 4).

There was no statistically significant difference in dormant buds between pre and post treatment for our treatments (Table 5).

There was a significant difference in dead buds between pre and post treatment for both the low and high-intensity treatments but not the control (Table 5). When comparing pre- and post-treatment values, the number of dead buds in the high-intensity treatment increased by 0.54±0.06 buds tiller^{-1} ($P < 0.0001$). Similarly, in the low-intensity treatment dead buds increased by 0.17±0.06 buds tiller^{-1} (*P* = 0.0063). In the control, dead buds were statistically unchanged pre- vs post-fire. Overall, fire treatments led to a significant increase in the number of dead buds (Fig. 4). The post-fire number of dead buds tiller^{-1} for our high- and low-intensity fire treatments differed with the high-intensity treatment having 0.36±0.08 more dead buds tiller^{-1} than the low-intensity treatment (*P* < 0.0001).

Table 5: Immediate (<24hr) fire intensity effects on the number of total, active, dormant, and dead belowground buds belonging to *H. belangeri* tillers. All bud means are given in buds tiller^{-1}. Means within bud classification are similar when followed by a common letter (*P*>0.05).

Bud Classification	Pre-treatment			Post-treatment		
	Control	Low	High	Control	Low	High
Total	2.8±0.1a	3.3±0.1c	3.3±0.1c	2.8±0.1ab	2.2±0.1bd	2.1±0.1d
Active	1.6±0.1a	1.8±0.1a	1.7±0.1ab	1.6±0.1a	0.6±0.1bc	0.2±0.1c
Dormant	1.2±0.1a	1.5±0.1a	1.6±0.1a	1.2±0.1a	1.5±0.1a	1.4±0.1a
Dead	0.01±0.1a	0.01±0.1a	0±0.1a	0.01±0.1a	0.2±0.1b	0.5±0.1c

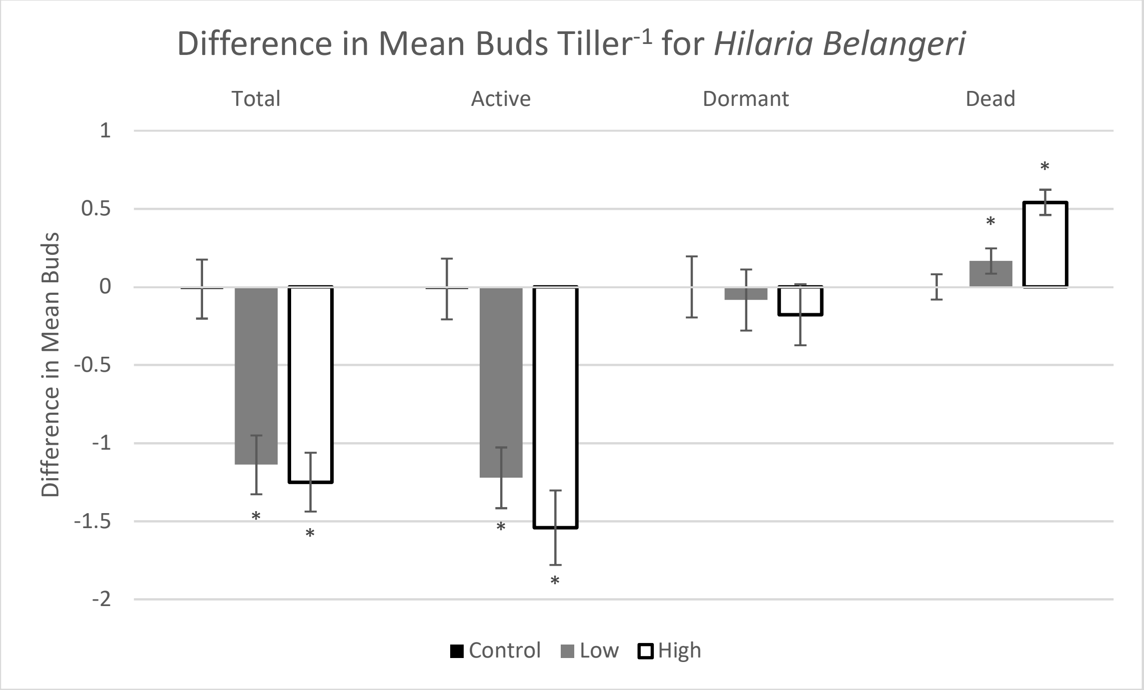

Figure 4: Difference in mean buds tiller^{-1} for *H. belangeri* between pre- and post-treatment values. Bars with an asterisk (*) indicate significant differences.

Bud depths

There were significant differences between *N. leucotricha* and *H. belangeri* in regards to mean bud depth (Mann–Whitney $U = 2720$, $n_1 = n_2 = 72$, $P < 0.0001$ two-tailed). On average, bud depth was 1.8cm for *N. leucotricha* and 0.5 cm for *H. belangeri* (Fig. 5).

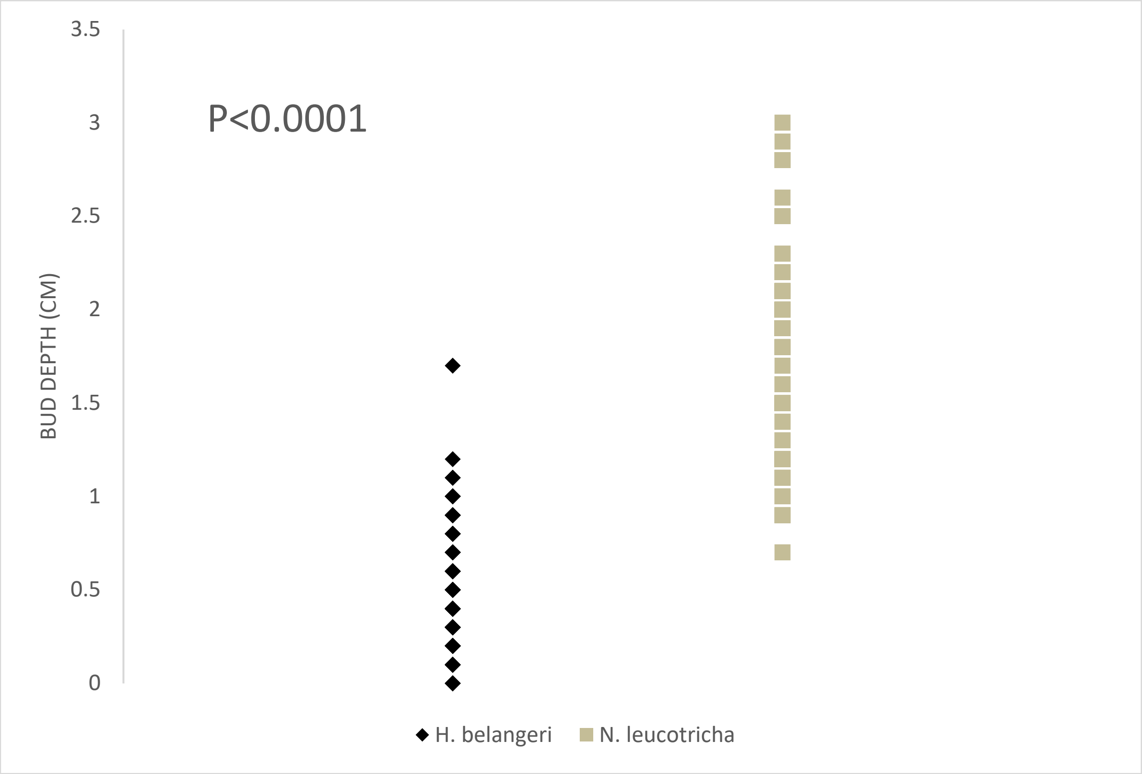

Figure 5: Bud depths in cm for *H. belangeri* and *N. leucotricha*.

Soil hydrophobicity

There was no significant effect of fire intensity, sampling period, or their interaction, on soil hydrophobicity. There was a slight increase in hydrophobicity pre vs. post-fire in the high-intensity treatment (increase of 0.2 sec), but this change was not significant. In both the low-intensity and control treatments, there was a decrease in hydrophobicity pre- vs. post-fire. However, similar to the high-intensity treatment, this decrease was not significant (Table 6).

Table 6: Time (seconds) for a water droplet to be absorbed by soil for each treatment 24 hr before and after treatment.

	Pre-treatment	Post-treatment
High	0.77±0.10	0.97±0.10
Low	0.94±0.10	0.69±0.10
Control	0.89±0.10	0.64±0.10

Initial reemergence

Three weeks after the burn, all *H. belangeri* individuals, with the exception of those in the high-intensity treatment, showed regrowth. In the high-intensity treatment, 75% of the marked individuals failed to resprout ($P < 0.0001$; Fig. 6). All marked *N. leucotricha* individuals resprouted for the control, low-intensity, and high-intensity treatments following the fires.

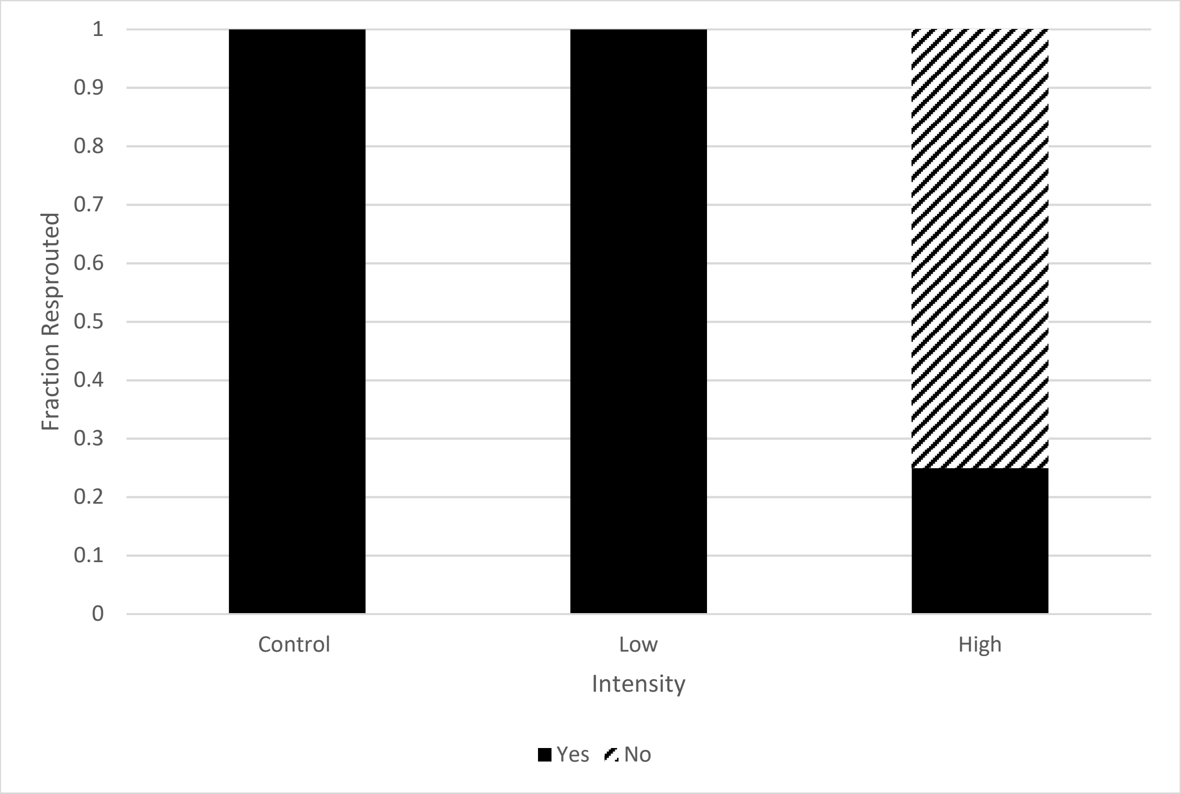

Figure 6: Fraction of *H. belangeri* individuals that did (yes=black) and did not (no=hatched) resprout 3 weeks following treatments (P < 0.0001).

Discussion

Immediate effects of fire intensity

Evaluation of the novel approach of using high-intensity fires as a management tool provides insight into the immediate effect of fire intensity on grass bud bank dynamics. Fire intensity had a significant effect on bud mortality for both grass species studied. For *N. leucotricha*, only high-intensity fires resulted in immediate increases in dead buds. However, for *H. belangeri*, both low and high-intensity fires resulted in significant bud mortality, with high-intensity fires increasing bud mortality more than low-intensity fires. Therefore, high-intensity fires have the ability to induce immediate bud mortality in these species, but the differences in the magnitude of bud mortality between species is likely mediated by growth form and photosynthetic pathway.

Fire intensity also had a significant effect on the number of active buds in *N. leucotricha*. Both high- and low-intensity fires led to a decrease in active buds, but high-intensity fires led to an even greater decrease. In contrast, *H. belangeri* experienced a significant decrease in active buds in both high- and low-intensity treatments, but this decrease was similar in both fire treatments. This suggests that fire intensity was not as important for *H. belangeri* in regards to changes in active buds. Therefore, similar to bud mortality, this difference between species may be due to growth form and phenology.

Growth form

Although we saw a direct effect of fire intensity on bud mortality, the number of dead buds post-fire were higher for *H. belangeri* as opposed to *N. leucotricha*. Interestingly, only *H. belangeri* experienced significant bud death following fires in the low-intensity treatment. This result is most likely an effect of differential growth forms that influence bud depth within the soil profile. In support, we saw a difference in bud depth between the two grass species, with *N. leucotricha* having deeper buds, on average, than *H. belangeri.* This is important because soil is considered an effective insulator (Valettel et al., 1994). As such, grass species whose buds are deeper in the soil profile are likely to experience lower temperatures than a grass with shallower buds. Therefore, fire intensity is likely more important for grasses with shallower bud banks, likely making bud depth an important determinant of grass survival following fires.

These findings suggest that resident abundant herbaceous growth forms should be considered before high-intensity fires are used as a management tool.

Previous studies have found that bunchgrasses are more susceptible to fire damage than other growth forms (Wright, 1971; Engle et al. 1998). In general, litter accumulates in the crown of these plants that increases the fuel load at the center of the plant, leading to greater heat duration and heat dosage (Wright, 1971; Engle et al. 1998) that potentially contributes to greater bud mortality closer to the center. Accumulation of litter over the years without frequent fires to remove plant detritus should only increase bud mortality. But this was not observed in our study, where *H. belangeri* was more susceptible to fire damage than *N. leucotricha*.

Haile (2011) suggests that this may be a result of the density of the bunch form, leading to less oxygen availability closer to the base of the plant. This would result in less heat transfer, decreased heat duration, and/or completely extinguishing the fire (Haile, 2011). Another potential explanation for this result is productivity. Bud banks increase in size on a precipitation gradient, with higher precipitation resulting in larger bud banks (Dalgleish & Hartnett, 2006). As such, higher precipitation should lead to higher aboveground productivity and larger plants. Wright and Klemmedson (1965) suggest that the size of a plant is important in determining the effect of fire on bunchgrasses, especially during the latter part of the summer. Therefore, with lower productivity in semi-arid grasslands and savannas, plants should not only be smaller but also generally have lower accumulation of litter resulting in less heat duration at the center of the plant.

Phenology

Another possible explanation for higher bud mortality in *H. belangeri* may be due to the timing of our burns. *Nassella leucotricha* is a C_3, cool-season grass. In contrast, *H. belangeri* is a C_4, warm-season grass. Due to these differences in functional groups, these species are likely to have differences in phenology relating to timing of increased bud activity or dormancy. Summer fires have been shown to favor C_3 over C_4 perennial grasses (Engle et al. 1998). These summer burns can damage warm-season, C_4 grasses because actively growing grasses are more easily damaged by fire than dormant grasses (Briske, 1991). On the other hand, C_3 grasses have finished most of their active growth by this time so the higher amount of dormant tissues may result in less damage by fire. However, despite *H. belangeri* being a C_4 grass, the control treatment did not have a large number of active buds during the timing of our burns. The amount of dormant and active buds was similar which may indicate that *H. belangeri* was not as active as we would expect which may be a result of increased stress due to our drought conditions. So, although functional group has a large influence on phenological patterns of bud growth and dormancy, it may not be as large as an impact as we expected nor fully explain the differences we saw.

Despite significant fire-induced bud mortality, the amount of bud death was relatively small. In the high-intensity treatment, approximately 17% of *N. leucotricha* buds died and approximately 20% of *H. belangeri* buds died. In the low-intensity treatment, approximately 7% of *H. belangeri* buds died. Even though these values seemed relatively small, they may have differentially impacted our species and resulted in the difference we saw in tiller reemergence following treatment. All permanently marked *N. leucotricha* individuals produced new tillers in the high-intensity treatment. This was not true for *H. belangeri*. Although not all *H. belangeri* buds died due to exposure to increased temperatures, we saw very few individuals produce new tillers 3 weeks following fire treatment. A possible explanation may be due to early dormancy and, therefore, delayed emergence. Since C_4 grasses become dormant during the fall and winter, our burns in early August may have induced dormancy earlier than usual due to increased stress by our high-intensity fire and drought conditions. Whether or not high-intensity fires impacted *H. belangeri* by inducing plant dormancy or by inducing plant death requires a longer-term study and an examination of fire seasonality effects on bud dynamics.

Immediate bud activation may not have occurred in either of our species due to similar reasons. Our fires were set during the summer and during low water availability, a time in which *N. leucotricha* should not be active because it is a C_3 grass. Therefore, bud activation may be delayed until later in the year. However, our C_4 grass is normally active during this time but drought along with the stress of aboveground tissue removal may lead to lower activity immediately following our treatments.

Soil hydrophobicity

There was no soil hydrophobicity found in our plots. The average time it took for a water droplet to penetrate our soil samples was less than a second, regardless of intensity. According to Steenhuis et al. (2001), this indicates a soil that is not water repellent. However, we did add a large amount of juniper limbs to the plots in order to create our high-intensity burns. This is important to note because burns in juniper woodlands have been shown to create instances of soil hydrophobicity (Madsen et al., 2011). Therefore, given our large additions of dried juniper, if soil hydrophobicity were to occur, it would logically have occurred in the high-intensity treatment.

A possible explanation is that the properties of the litter available for fire consumption in our plots do not contain high amounts of hydrophobic compounds. Cesarano et al. (2016) found moderate repellency associated with two perennial grasses but, when considering aged litter, found that water repellency decreased substantially after 180 days. Because our site is predominantly covered by perennial grasses, it is unlikely that the litter contains high amounts of hydrophobic compounds that may produce soil hydrophobicity naturally or following a fire.

Another pertinent factor to consider is that the study by Ceserano et al. (2016) was done in sandy soils. In fact, Bond (1969) suggests that low clay content soils are naturally water repellent. On top of this, Bond (1969) suggests that sandy soils became more readily water repellent following burning than soils with higher clay contents. Our soils have a high clay content and that may have aided in resisting the formation of a hydrophobic layer in the high-intensity treatment.

In this experiment, it is important to note that the high-intensity fires were ground fires that turned into crown fires and not crown fires alone. These fires were not reminiscent of natural high-intensity fires set by Twidwell (2016). Therefore, we cannot directly compare the results of these two studies. Even the low-intensity fires were uncharacteristic given the added hay in order to achieve a continuous burn.

Conclusions

Most grasses primarily reproduce clonally via a belowground bank of axillary buds. As such, it is important to understand bud bank dynamics of these grasses in order to discern dynamics of annual regeneration, especially when impacted by disturbances, such as fires. With no evidence of hydrophobic soil layers, our immediate bud responses are most likely results of differences in phenology and growth forms. C_3 and C_4 grasses are active during different times of the year and bunchgrasses and stoloniferous grasses may be differentially affected by fire intensity. This suggests the need for managers to consider both growth form and photosynthetic pathways in determining grass survival following high-intensity fires during low water availability.

With the use of high-intensity fires during drought as a management tool to remove invasive shrubs, this study serves to assuage some fears in relation to these extreme fires. In areas dominated by perennial grasses and with soils with high clay content, it is unlikely for hydrophobic layers to be created in response to high-intensity fires. Similarly, high-intensity fires may cause immediate bud death, but, in the case of *N. leucotricha*, many dormant buds survived and new tillers were produced a couple weeks following treatment. In the case of *H. belangeri*, high-intensity fires had a greater impact and most likely suppressed regrowth due to differences in physiology. Therefore, high-intensity fires may suppress some grass species and potentially alter community dynamics.

Longer-term analyses and a comparison of fire effects during different times of the growing season will increase our understanding of these dynamics. Fire effects can be seen over an extended period of time, so recovery of the bud bank and potential legacy effects need to be researched. On top of this, the effect of high-intensity fires on the microbial community and soil nutrients need to be investigated to understand how these integral components of the ecosystem may affect graminoid recovery.

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CHAPTER III

PHENOLOGY AND RECOVERY OF NATIVE PERENNIAL GRASS BUD BANKS FOLLOWING HIGH-INTENSITY FIRE IN A SEMI-ARID SAVANNA SYSTEM

Chapter Summary

Aboveground growth and production of native perennial grasses is determined by vegetative reproduction from belowground bud banks. Despite their importance, the phenology and dynamics of these belowground bud banks are poorly researched, even for dominant grass species. This information becomes even more essential when considering the potential use of management tools, such as high-intensity fires to remove invasive woody shrubs, which may impact herbaceous bud bank dynamics. Patterns of belowground bud activity, dormancy, and mortality of two dominant perennial grasses were investigated: *Nassella leucotricha* ($C_{3,}$ caespitose growth form) and *Hilaria belangeri* (C_4, stoloniferous growth form). Our treatments were applied in the summer and consisted of no burn (control), low-intensity burn, and the high-intensity burn plots. In both low-intensity fire and control treatments, dormant buds increased during the winter and total buds remained relatively stable through the year for *H. belangeri*. On the other hand, *N. leucotricha* maintained a smaller dormant bud bank through the winter, with larger fluctuations in total buds. In high-intensity burned treatments, *N. leucotricha* recovered; bud counts being comparable to the low-intensity and control treatments eight months following treatment. However, at this time, *H. belangeri* did not return to pre-treatment bud totals in high-intensity burned treatments. These differing bud bank responses to fire intensity may be a result of the differential phenology and growth forms of our two target species. Understanding the influence of these factors on tiller recruitment from the bud bank will allow better predictions of bud development in response to various management tools, such as high-intensity fires.

Keywords: axillary bud, bud bank, dormancy, extreme fire, savanna, tiller, vegetative reproduction

Introduction

In grassland and savanna communities dominated by perennial grasses, aboveground growth following disturbances is often determined by regrowth from a belowground bud bank (Benson and Hartnett, 2006; Dalgleish and Hartnett, 2009). This type of vegetative growth is prolific, with estimates of more than 99% of all new tiller growth originating from belowground buds (Benson and Hartnett, 2006). Given the ubiquity and importance of vegetative reproduction in graminoids, bud bank phenology is still poorly researched. For most native grasses, belowground stages of bud development are unknown.

Understanding the phenology of buds is important because bud bank dynamics determine bud bank size through patterns of bud activation, dormancy, and mortality (Ott and Hartnett, 2011). In turn, bud bank size determines the growth potential of perennial grasses because it directly relates to a plant's ability to react to pulses of resource availability and respond to disturbances (Dalgleish and Hartnett, 2006; Russell et al., 2013). For example, species with extensive belowground bud banks are able to capitalize on increases in resource availability, such as nitrogen following fire or precipitation events (Dalgleish and Hartnett 2006). On the other hand, species that are meristem limited are unable to respond to pulses in resource availability and often cannot completely replace the aboveground tiller population following disturbance or senescence (Dalgleish and Hartnett, 2006). Given these potential restrictions, bud bank size is a key component in community response to disturbances in grassland and savanna systems.

Patterns of phenology are dependent on growth forms, photosynthetic pathway, and can be altered by timing and intensity of fires. Grass growth forms (e.g., stoloniferous vs. caespitose) are influenced by patterns of belowground bud development and are largely a result of differential patterns of bud placement and subsequent outgrowth (Briske, 1991; Hendrickson and Briske, 1997). As such, grasses with different growth forms should have different bud bank phenology and should be differentially affected by timing and intensity of fires.

On the other hand, grass photosynthetic pathway types (e.g., C_3 vs. C_4) can have a large influence on belowground bud development due to differences in phenology (Briske, 1991). Photosynthetic pathway type determines optimal growing temperatures, reproductive phenology, and geographic distribution (McIntyre, 1967). Because of this, photosynthetic pathway should strongly influence belowground bud bank development, and, therefore, temporal changes in bud activity and dormancy (Ott and Hartnett, 2012).

C_3 and C_4 species should show different bud bank phenology due to their differing time of active growth.

C_4 grass species have been shown to maintain large bud banks and their associated buds tend to live multiple years (Benson, Hartnett, and Mann, 2004; Ott and Hartnett, 2012; Ott and Hartnett, 2015a). As a direct result, tiller recruitment comes from buds originating from different cohorts rather than a single cohort (Benson, Hartnett, and Mann, 2004; Ott and Hartnett, 2012; Ott and Hartnett, 2015a). C_3 grasses, on the other hand, maintain smaller bud banks and their associated buds usually survive for a single year (Benson, Hartnett, and Mann, 2004; Ott and Hartnett, 2012; Ott and Hartnett, 2015a). Given these differences, buds seem to play different roles in the growth dynamics of C_3 compared to C_4 grass functional groups (Benson, Hartnett, and Mann, 2004; Ott and Hartnett, 2012; Ott and Hartnett, 2015a).

In the Edwards Plateau ecoregion of Texas, woody encroachment has become an extensive problem due to the removal of fires and increase in grazing intensity (Scholes and Archer, 1997; Bond, 2008; Allred et al., 2012). Previous works have shown that the use of high-intensity fires during drought can be used effectively to achieve high woody shrub mortality when attempting to restore herbaceous dominated savanna ecosystems (Twidwell et al., 2016). Few studies have shown the effect of such high-intensity fires on the graminoid community and their belowground bud banks. Therefore, understanding how fire intensity relates to bud bank phenology will help determine the impact of fire intensity on belowground and aboveground recovery.

Understanding perennial bud bank phenology will lead to better prediction of tiller recruitment following aboveground disturbances. In particular, understanding bud phenology and how it relates to growth form and photosynthetic pathways will allow predictions of survival and regrowth following high-intensity fires used as a management tool. The objectives of this study were to 1) assess bud development dynamics of two perennial grasses in a semi-arid savanna, 2) determine the impact of fire intensity and timing of fire on the recovery of these grasses over the course of 8 months following treatment, and 3) compare and contrast bud bank dynamics responses to different fire intensities relative to the different photosynthetic pathways types and growth forms (e.g., C_3 caespitose vs C_4 stoloniferous) of the two grass species.

Materials and Methods

Site description

The study site was located at the Sonora Texas A&M Agrilife Research Station (SARS) located in the Edwards Plateau ecoregion in Texas (-100.574°, 30.251°). The site is a semi-arid, savanna system that experiences a bimodal precipitation pattern, with rainfall peaking in the spring and fall (Fig. 7). The average annual precipitation ranges from 356 to 889 mm while the average annual temperature ranges from 14 to 21 ˚C, with summer temperatures reaching up to 38 ˚C. The western Edwards Plateau ecoregion experiences a fire return interval of 1-12 years (Stambaugh et al. 2014). Historically, fires were common during late winter and late summer when lightning strike frequency was high (Stambaugh et al. 2014).

The soils at our site are in the Tarrant soils (Clayey-skeletal, smectitic, thermic Lithic Calciustolls; USDA, 2016). The soils are shallow, often with exposed limestone bedrock. The dominant vegetation consists of isolated clumps of trees within a matrix of graminoids. The dominant trees in the area are *Quercus spp.*, *Juniperus spp.*, and *Prosopis glandulosa* Torr.. The dominant graminoid species are *Hilaria belangeri* (Steud.) Nash, *Aristida spp*., *Bouteloua curtipendula* (Michx.) Torr., *Nassella leucotricha* (Trin. & Rupr.) R.W. Phol, and *Pleuraphis mutica* Buckley.

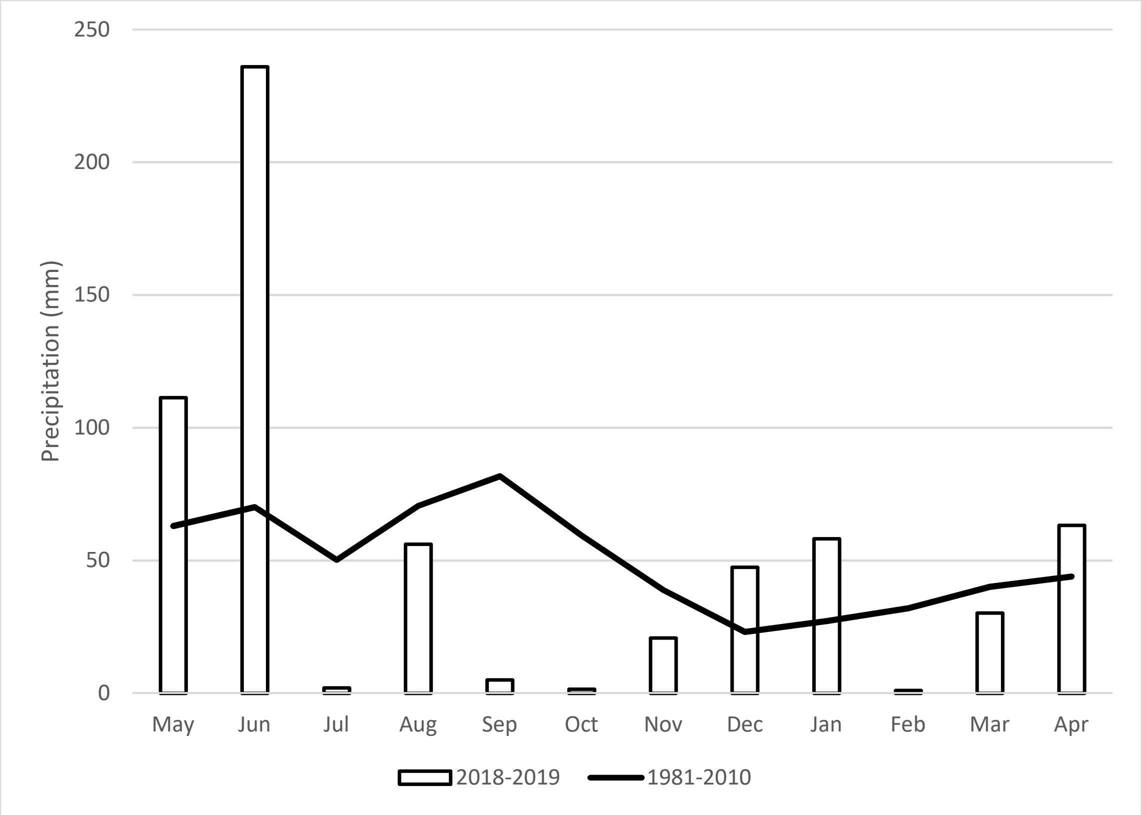

Figure 7: Study site mean precipitation.

Experimental design and fire measurements

A total of 36 plots, each 100 m^2, were demarcated and centered on a mature mesquite shrub that was a minimum of 10 yr old and 3-5 m in height. The study site was last burned in a high-intensity prescribed fire in August of 2000. The study site historically was moderately grazed by sheep and goats but was rested at least one growing season prior to our burns. Each plot was randomly assigned to one of three fire treatments: i) no burn (control), ii) low-intensity, and iii) high-intensity.

Two grass species were selected for focus in this study due to their abundance and dominance at the site, along with their contrasting photosynthetic pathways and growth forms: *N. leucotricha* (C_3, caespitose) and *H. belangeri* (C_4, stoloniferous).

With each large 100 m^2 plot, two smaller, mini-plots of 1 m^2 each were demarcated with rebar. Each mini plot was created around a patch of either *N. leucotricha* or *H. belangeri*. Therefore, each large plot had one *N. leucotricha* mini plot and one *H. belangeri* mini plot. These mini-plots served as a reference group for tiller collections described in the next section.

In the early spring of 2018, the entire pasture, besides our experimental plots, was burned to reduce fuel loads in the surrounding area. A 5m fire-break was bulldozed around each plot. The low-intensity fire plots required dry hay to be added to produce a continuous, low-intensity fireline. High-intensity fires were achieved by adding dried juniper branches along with hay. Fuel addition averages are and total fuel consumed averages are reported for each treatment in Table 7. Each plot was burned using the ring fire method between the dates of July 30th and August 4th, 2018. Energy and residence time of our fires were calculated using a FLIR (infrared) camera situated from an oblique position from each plot on a boom lift (Table 8). The residence times given are relative residence times and do not necessarily correspond to flame residence times.

Table 7: Fuel additions and consumption at the plot level for low-intensity and high-intensity treatments.

	Mean ± SE	**Maximum**	**Minimum**
Hay Addition (kg)	61 ± 1	96	47
Juniper Addition (kg)	201 ± 1	218	197
Fuel consumed (low-intensity)	55 ± 1	80	46
Fuel consumed (high-intensity)	259 ± 3	294	243

Table 8: FLIR (infrared) data measurements at the mini plot level for low and high-intensity treatment plots. Numbers are averages based on 11 low-intensity plots and 12 high-intensity plots.

	Mean ± SE
Average energy of low-intensity plots (KJ/m^2) [1]	42±5
Average energy of high-intensity plots (KJ/m^2) [1]	1392±283
Residence time of low-intensity plots (s) [2]	29±3.8
Residence time of high-intensity plots (s) [2]	809±152.7

[1] Energy of grass plot integrated over the period in which radiation was greater than or equal to ½ of the maximum (full width at ½ maximum, FWHM) radiation
[2] Residence time calculated as the time between the first rise and first fall below FWHM power

Sampling

From May to August (2018), tillers were harvested from three randomly determined individuals of each species inside each large plot. From September 2018 to March 2019, due to decreased time availability, the number of individuals collected per plot was reduced to two for each species. Two tillers were collected and analyzed per individual. All tillers were collected from individuals in similar phenological stages as those in the permanent mini-plots using the classification system of Moore et al. (1991). Plants visibly damaged by herbivores, insects, or pathogens were excluded.

Tillers were harvested every two weeks during the growing season and once a month during dormancy. Harvesting consisted of removing 3-6 tillers per individual, using a trowel to keep both belowground and aboveground structures intact. Only two tillers were used for analysis but more were harvested in case some tillers were unusable.

The buds associated with each tiller were counted and their activity classified as either active, dormant, or dead using the Tetrazolium and Evans Blue staining procedures laid out by Busso (1989). To determine activity, tillers were submerged in a Tetrazolium solution for 24h at room temperature. Active buds were stained pink/red while dormant and dead buds retained a white/yellowish color. Each tiller was then submerged in Evan's Blue solution for 20 min to determine the activity of dormant and dead buds. If buds were dormant, they retained their white pigmentation, while dead buds were stained a dark blue.

Statistical analyses

Bud response data were analyzed using ANOVAs (MIXED procedure of SAS, Littell et al., 2006). The model included date, fire intensity treatment (no burn, low-intensity, high-intensity), and their interaction as fixed effects. The analyses were done by species, with total, active, dormant, and dead buds as response variables. Statistical significance was set at $P < 0.05$.

Results

Nassella leucotricha dynamics

Total bud numbers peaked, for all treatments, in May ($P < 0.05$), decreased until December, and increased thereafter (Fig. 8). Before treatment application in July, all treatments were statistically similar ($P > 0.05$). The main effects, treatment and date, were significant ($P < 0.0001$) but the interaction between the two was not ($P = 0.0577$).

Total bud numbers in the high-intensity fire treatments were significantly lower than the control for up to 4 months following treatment (Aug-Nov). Following this period, and other than in January (6 months after treatment), total bud numbers in high-intensity treatment were not different from those in the control.

Total bud numbers in the high-intensity treatment were not significantly different from the low-intensity treatment for the data collection period, except for early September, January, and March (respectively 1.5 months, 6 months, and 8 months following treatment) ($P < 0.05$). The total number of buds in the high-intensity treatment were consistently lower than in low-intensity treatment (Fig. 8).

There were no significant differences between low-intensity treatment and control in regards to total bud numbers (Fig. 8).

The highest total buds per tiller for all intensities was in May, with high-intensity, low-intensity, and control having 3.5±0.1, 3.4±0.1, and 3.8±0.1 buds per tiller respectively (Fig. 9). The lowest buds per tiller for low and control occurred in late November and the lowest buds per tiller for the high-intensity treatment was in December (Fig. 9). The average minimum bud count per tiller was 0.7±0.1 for high-intensity treatment, 0.8±0.1 for low-intensity treatment, and 1±0.1 for control (Fig. 9). The minimum, maximum, and timing of each of these were very similar between high-intensity treatment, low-intensity treatment, and control.

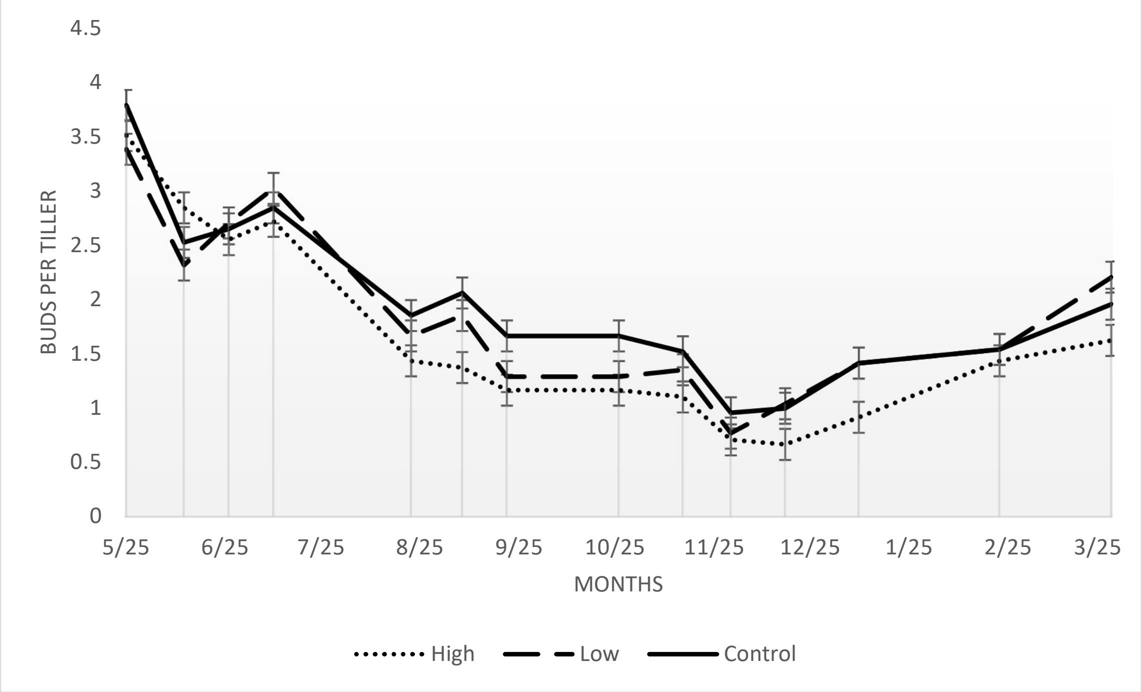

Figure 8: Total buds per tiller in high-intensity treatment, low-intensity treatment, and control over the year for *N. leucotricha*.

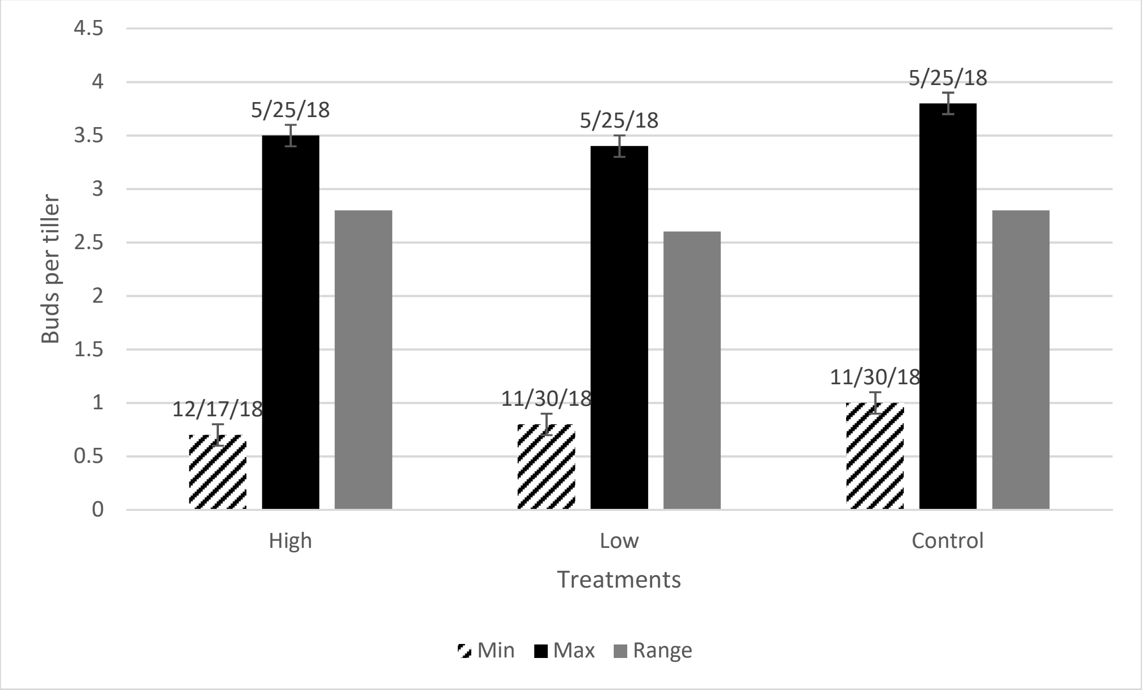

Figure 9: The minimum, maximum, and range of total buds per tiller for *N. leucotricha*. Dates above bars indicate the date in which the max or minimum was reached.

Active bud numbers peaked, for all fire intensities, in May (P < 0.05) and had an insignificant but trending peak in July (Fig. 10). Before treatment, all treatments were statistically similar ($P > 0.05$). Overall, the main effects, treatment and date, were significant ($P = 0.0003$; $P < 0.0001$) but the interaction between the two was not ($P = 0.2526$).

Active bud numbers in the high-intensity treatment were not significantly different from the control treatment immediately following treatment but were significantly different for 2 months following August measurements ($P < 0.05$). For these months, active bud numbers were consistently lower for the high-intensity treatment than the control treatment (Fig. 10).

Active bud numbers in the low-intensity treatment were smaller than in the control treatment for the 1.5 months following late September measurements ($P < 0.05$; Fig. 10). There were no differences between high- and low-intensity treatments in regards to the number of active buds.

The highest active buds per tiller for all intensities was in May, with high-intensity treatment, low-intensity treatment, and control having 3.0±0.1, 3.0±0.1, and 3.3±0.1 buds per tiller respectively (Fig. 11). The lowest active buds per tiller for all intensities was in December, with the high-intensity treatment, low-intensity treatment, and control treatment having 0.4±0.1, 0.5±0.1, and 0.5±0.1 buds per tiller respectively (Fig. 11). The minimum, maximum, and timing of each of these were very similar between high-intensity, low-intensity, and control treatment.

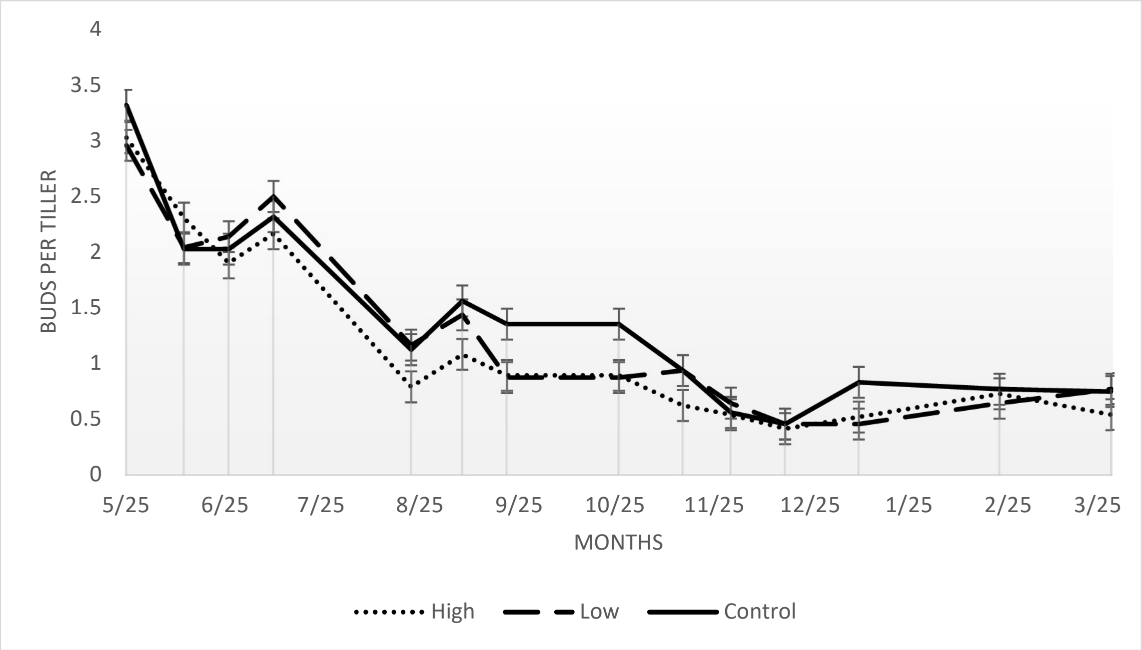

Figure 10: Active buds per tiller in high-intensity, low-intensity, and control treatment over the year for *N. leucotricha*.

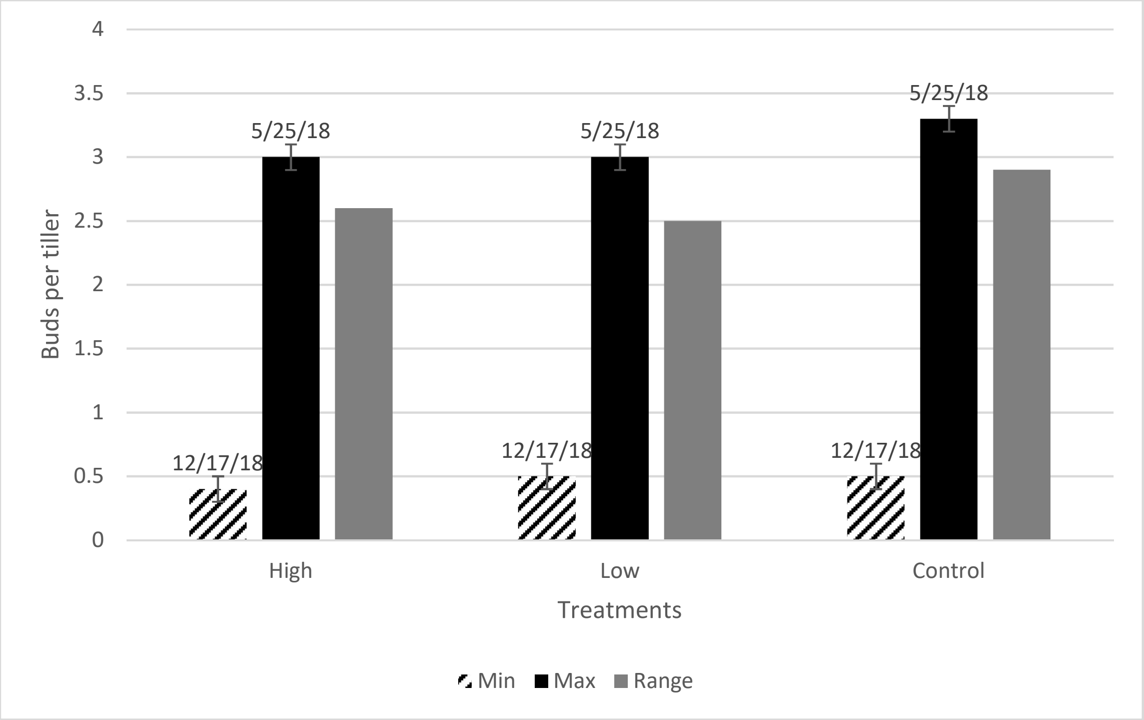

Figure 11: The minimum, maximum, and range of active buds per tiller *N. leucotricha*. Dates above bars indicate the date in which the max or minimum was reached.

During most of the season, the number of dormant buds per tiller remained relatively low and constant before increasing from November onwards (Fig. 12). In comparison, active buds gradually declined over the season. Prior to treatment application, all treatments were statistically similar ($P > 0.05$). Overall, the main effects, treatment and date, were significant ($P = 0.0059$; $P < 0.0001$) but the interaction between the two was not ($P = 0.1504$).

Dormant bud numbers in the high-intensity treatment were lower than in the control for a month following treatment ($P < 0.05$; Fig. 12). Dormant bud numbers in the high-intensity treatment were significantly lower than those in the low-intensity treatment for the month of January (6 months post-treatment) ($P < 0.05$; Fig. 12). Dormant bud numbers in the low-intensity treatment were significantly higher than those in the control for the month of January (6 months post-treatment) ($P < 0.05$; Fig. 12).

The highest occurrence of dormant buds per tiller for all treatments was in March, with the high-intensity, low-intensity, and control treatment having 1.1±0.1, 1.4±0.1, and 1.2±0.1 buds per tiller respectively (Fig. 13). The lowest dormant buds per tiller for the high- and low-intensity treatment occurred in late November and the lowest occurrence of dormant buds per tiller for the control was in May (Table 10). The average minimum bud count per tiller was 0.2±0.1 for the high-intensity treatment, 0.1±0.1 for the low-intensity treatment, and 0.2±0.1 for the control (Fig. 13). The minimum, and the time at which the maximum occurred, was similar between the high-intensity, low-intensity, and control. However, the low-intensity maximum was considerably larger than the maximum for the high-intensity plots.

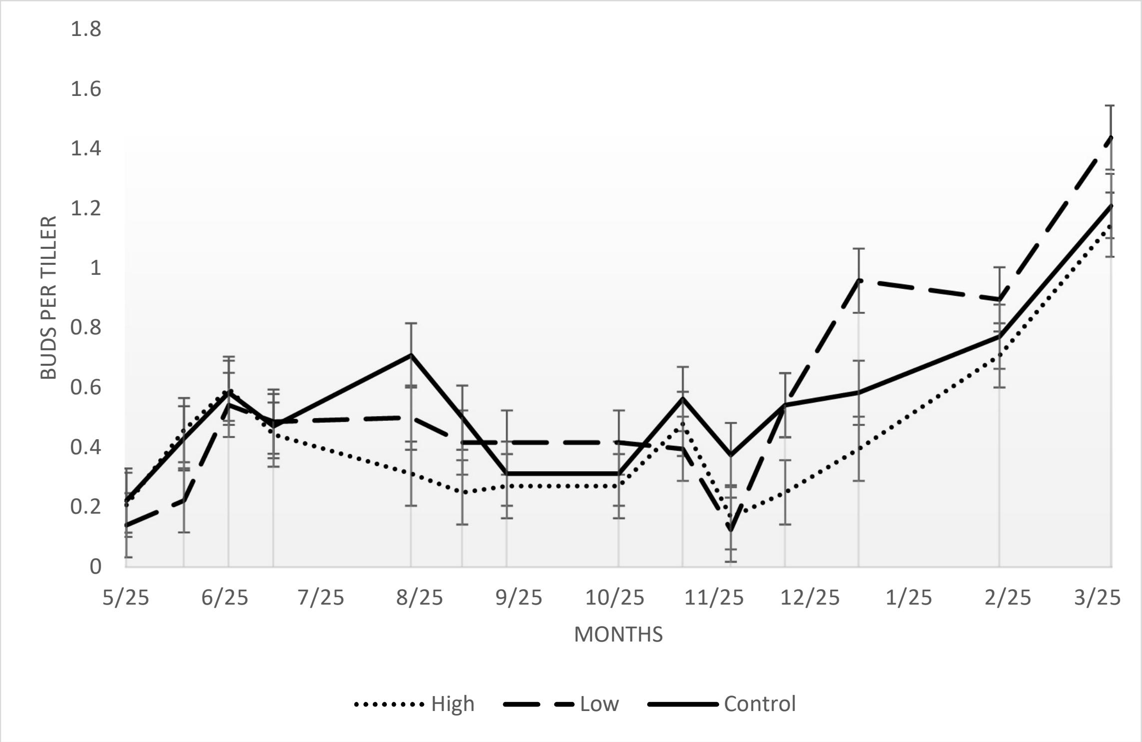

Figure 12: Dormant buds per tiller in the high-intensity, low-intensity, and control treatment over the year for *N. leucotricha*.

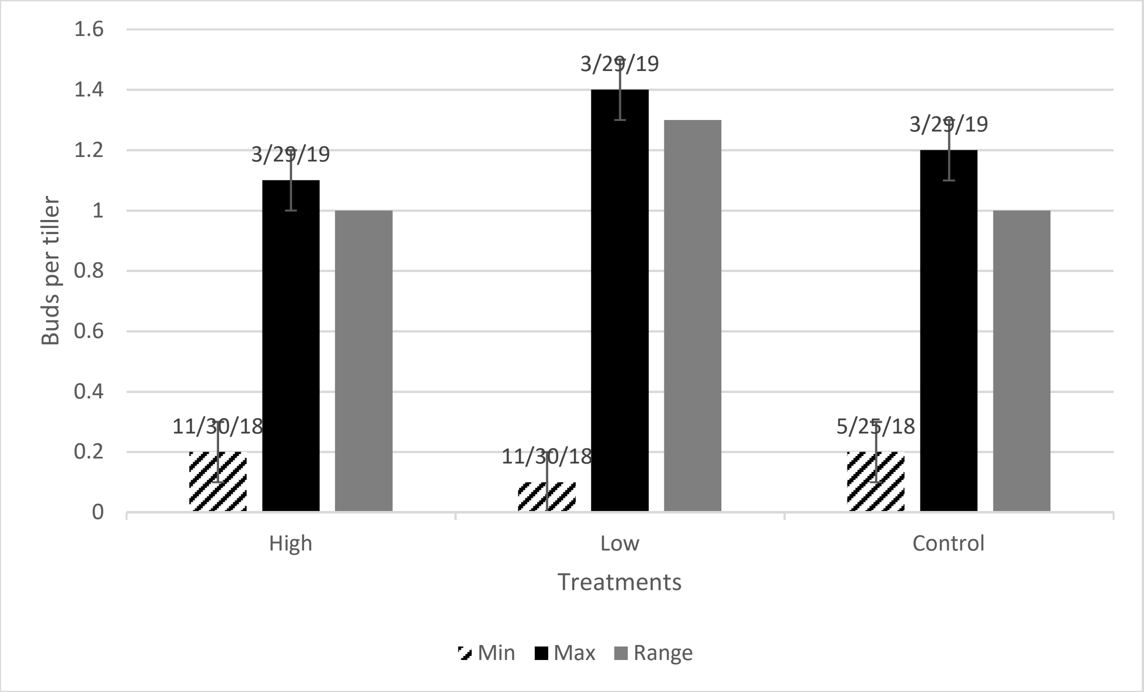

Figure 13: The minimum, maximum, and range of dormant buds per tiller for *N. leucotricha*. Dates above bars indicate the date in which the max or minimum was reached.

Dead bud numbers, for all treatments, peaked in May ($P < 0.05$; Fig. 14). However, for the high-intensity treatment, dead bud numbers peaked again 1 month following treatment ($P < 0.0001$; Fig. 14). Despite these peaks, dead bud numbers remained relatively low over the course of the season. Prior to treatment application, all treatments were statistically similar ($P > 0.05$). Overall, the main effects, treatment and date ($P = 0.0279$; $P < 0.0001$), and the interaction between the two was significant ($P = 0.0026$).

The only significant difference in dead bud numbers between treatments was 1 month following treatment, where the high-intensity treatment had a significant increase in dead buds as opposed to the low-intensity treatment and control ($P < 0.0001$).

The highest dead buds per tiller for the low-intensity treatment and control was in May, while the greatest dead buds per tiller for the high-intensity treatment was 1 month following fire treatment (Fig. 15). For all treatments, the average maximum of dead buds per tiller was 0.30±0.04 and the lowest dead buds per tiller was 0.00±0.04 (Fig. 15). The minimum and maximum dead buds per tiller were very similar between the high-intensity, low-intensity, and control treatment. However, the time at which the maximum dead buds per tiller was reached was different between the high-intensity treatment and the low-intensity and control treatment.

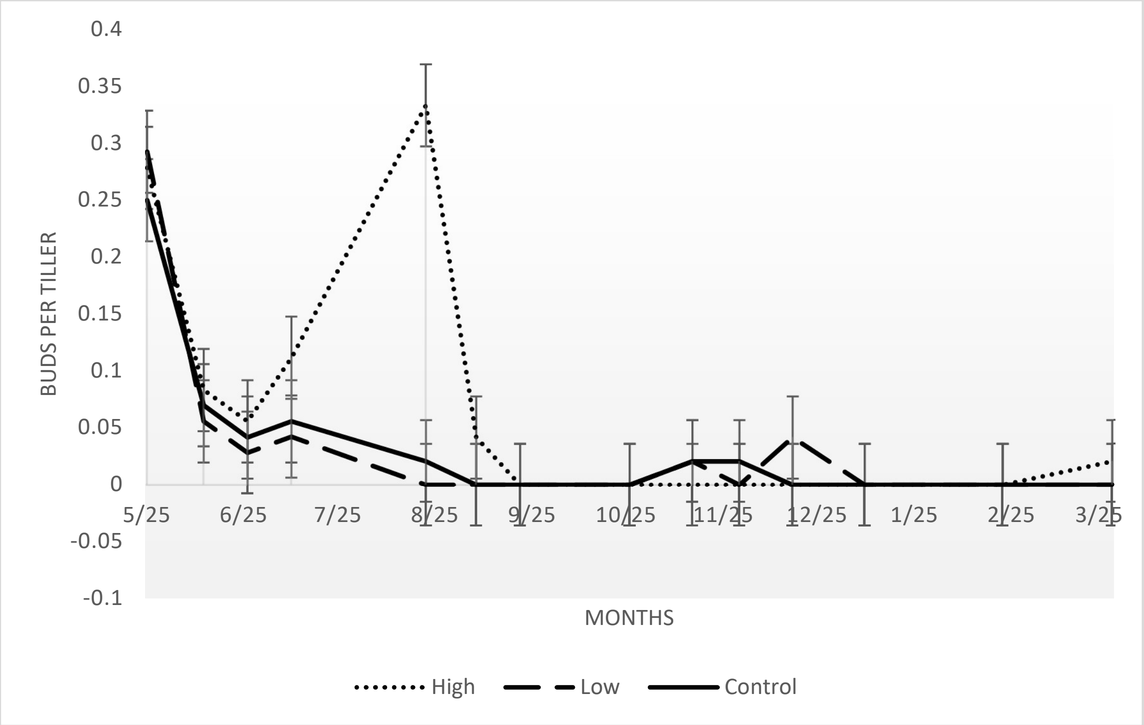

Figure 14: Dead buds per tiller in the high-intensity, low-intensity, and control treatment over the year for *N. leucotricha*.

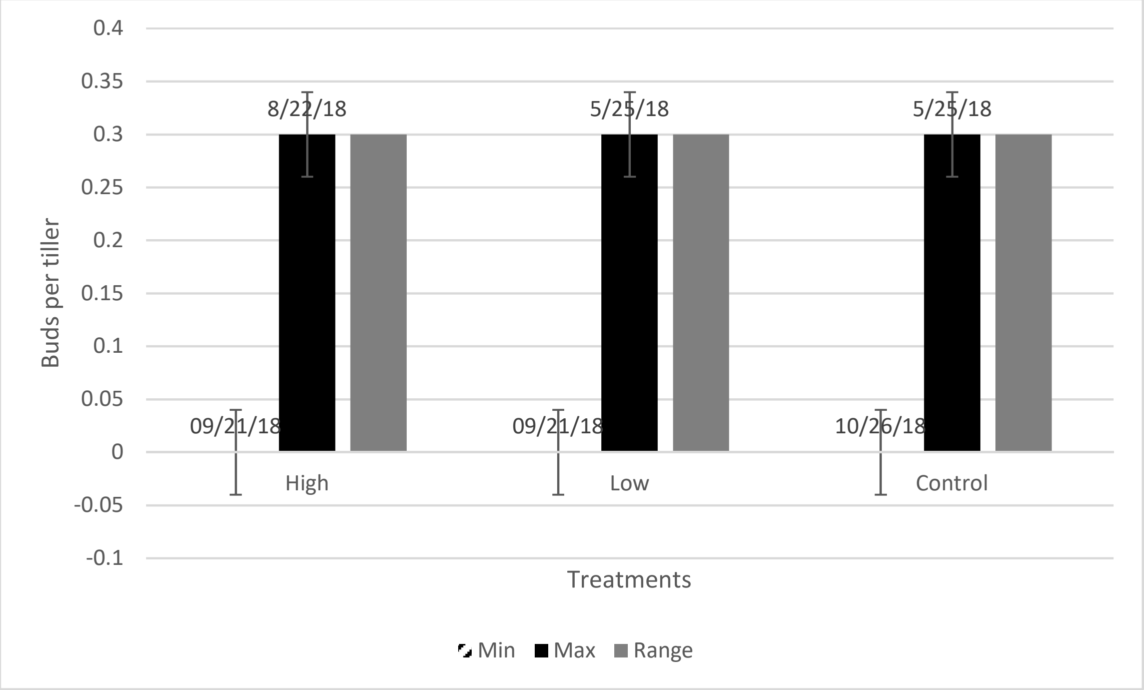

Figure 15: The minimum, maximum, and range of dead buds per tiller for *N. leucotricha*. Dates above bars indicate the date in which the max or minimum was reached.

Hilaria belangeri dynamics

Total bud numbers remained relatively consistent through the season for all treatments, with small, insignificant peaks in early June, July, December (Fig. 16). The control had a single significant peak in February ($P < 0.05$). The only exception to these trends was in the high-intensity treatment in which there was a sharp decline in total bud numbers from treatment application up to 1.5 months following treatment. Following this period, no buds were recorded for the rest of the year except for a small increase in February, 7 months following treatment. Zero buds were recorded for a large portion of the year because most of the *H. belangeri* individuals in the high-intensity treatment failed to recruit new tillers after late September.

Overall, the main effects, treatment and date, and the interaction between the two, were significant ($P < 0.0001$). There were significant differences in total bud numbers between the high-intensity treatment and both the low-intensity and control treatment for 8 months following treatment ($P < 0.05$; Fig. 16). Both the low-intensity and control treatment had greater total bud numbers than the high-intensity treatment following treatment (Fig. 16).

The only difference in total bud numbers found between the low-intensity treatment and the control was in early September (1.5 months post-treatment) ($P = 0.0094$; Fig. 16). During this month, total bud numbers were lower in the low-intensity treatment than in the control (Fig. 16).

The greatest total buds per tiller for high- and low-intensities was in early June, and the greatest total buds per tiller for the control was in February (Fig. 17). The average maximum bud count per tiller was 3.5±0.2 for the high-intensity treatment, 3.2±0.2 for the low-intensity treatment, and 3.3±0.2 for the control. The lowest total buds per tiller for the high-intensity treatment was 0.0±0.2, and this first occurred in late September. The lowest total buds per tiller for the low-intensity treatment was in early September and the lowest total buds per tiller for the control was in early November (Fig. 17). The average minimum total bud per tiller was 1.7±0.2 for the low-intensity treatment and 1.9±0.2 for the control. The low-intensity and control treatments were very similar in regards to minimum and maximum total buds per tiller, along with timing of these statistics. However, the high-intensity deviated largely from the low-intensity and control treatments in regards to minimum total buds per tiller.

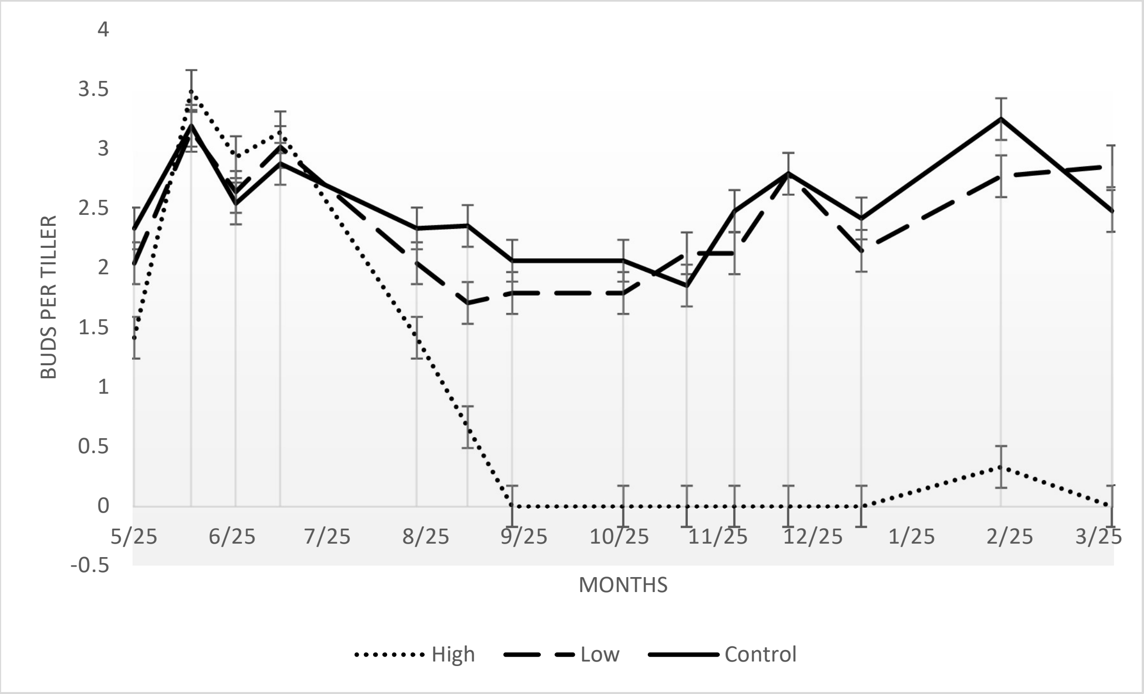

Figure 16: Total buds per tiller in the high-intensity, low-intensity, and control treatments over the year for *H. belangeri*.

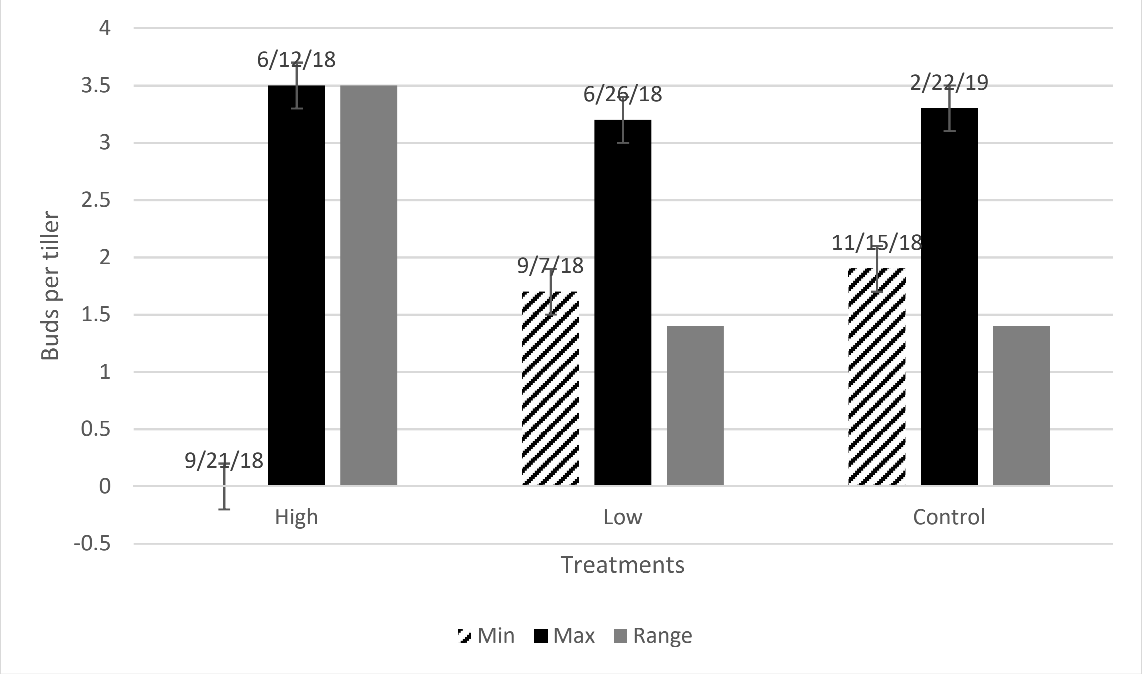

Figure 17: The minimum, maximum, and range of total buds per tiller for *H. belangeri*. Dates above bars indicate the date in which the max or minimum was reached.

Active bud numbers were the highest, for all fire intensities, in May ($P < 0.05$; Fig. 18). From May onward, active buds decreased over time until increasing incrementally from January to March. The high-intensity treatment did not follow this trend. Prior to treatment application, all treatment were statistically similar ($P > 0.05$). Overall, the main effects, treatment and date, and the interaction between the two, was significant ($P < 0.0001$).

Active bud numbers in the high-intensity treatment were lower than those in the control and low-intensity treatments for up to 5 months following treatment, and again in March (8 months post-treatment) ($P < 0.05$; Fig. 18).

Active bud numbers in the low-intensity treatment were not different immediately following treatment. However, for 2.5 months following August measurement, the active bud number in the low-intensity treatment were lower than the control (*P* < 0.05; Fig. 18).

The greatest active buds per tiller for all treatments was in May, with the high-intensity, low-intensity, and control treatments having 3.1±0.1, 3.2±0.1, and 3.1±0.1 buds per tiller respectively (Fig. 19). The lowest active buds per tiller for the high-intensity treatment was in late September, for the low-intensity treatment was in January, and for the control was in late November (Fig. 19). The average minimum active bud count was 0±0.1 for the high-intensity treatment, 0.1±0.1 for the low-intensity treatment, and 0.2±0.1 for the control. The minimum and maximum dead buds per tiller, and the timing of these statistics, were very similar between our treatments.

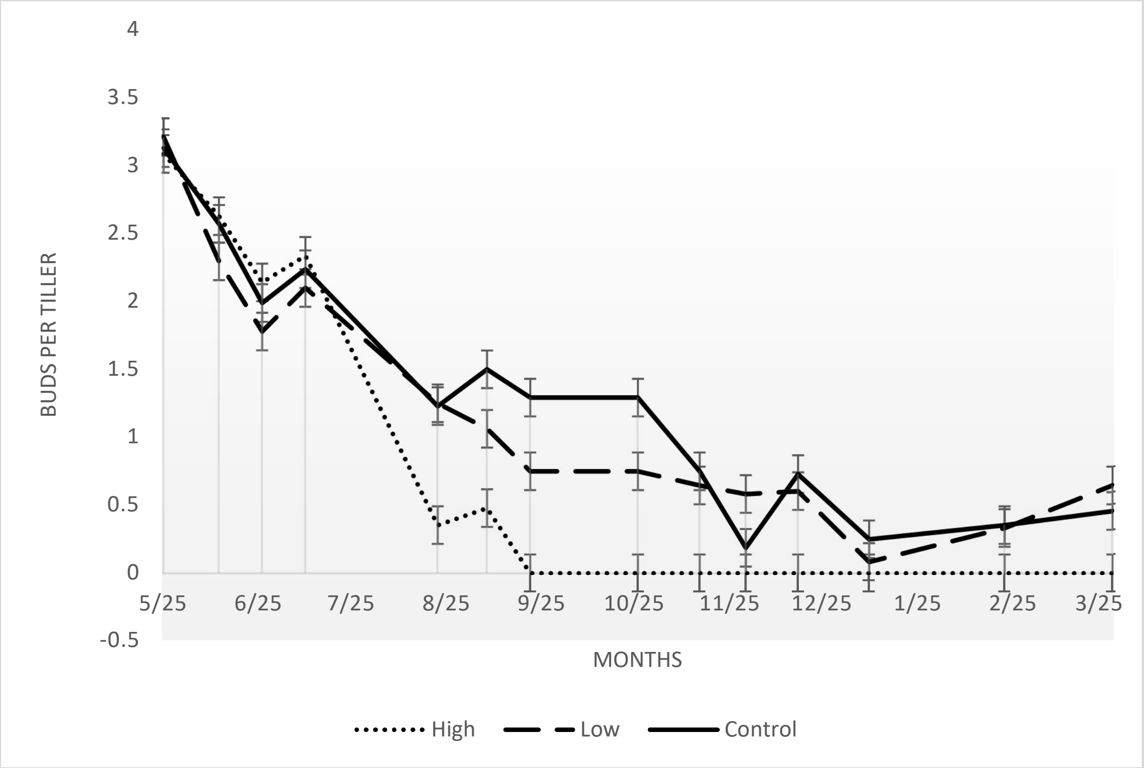

Figure 18: Active buds per tiller in the high-intensity, low-intensity, and control treatments over the year for *H. belangeri.*

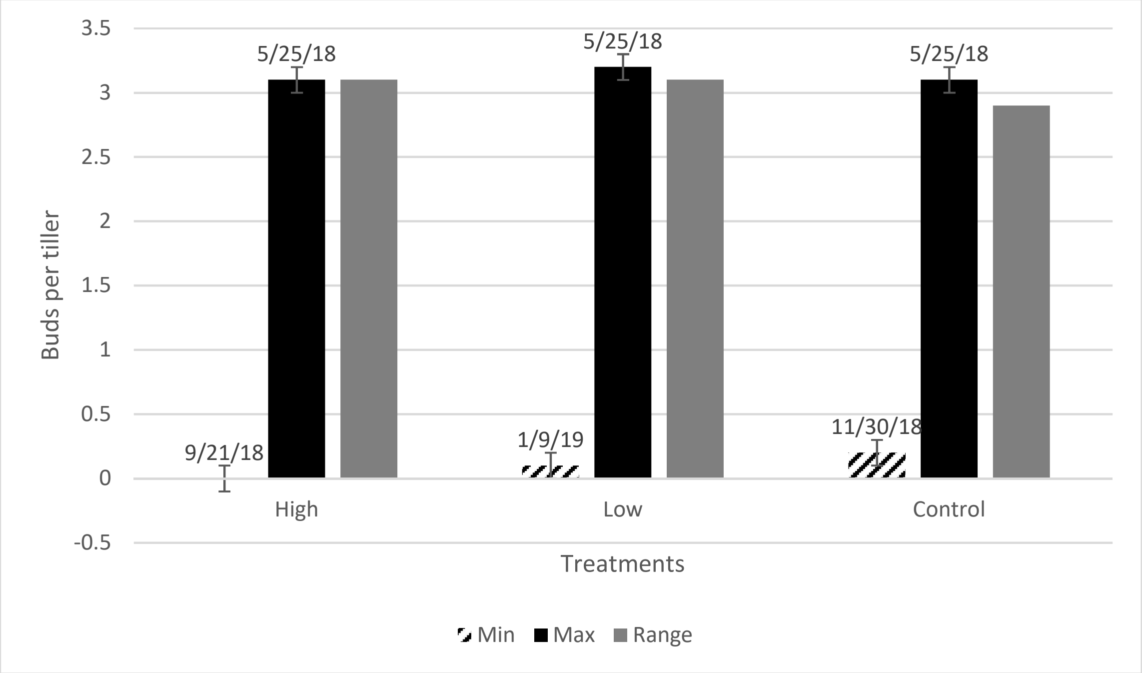

Figure 19: The minimum, maximum, and range of active buds per tiller for *H. belangeri*. Dates above bars indicate the date in which the max or minimum was reached.

Dormant bud numbers began very low in May and steadily increased over the season for both the low-intensity and control treatments (Fig. 20). The high-intensity treatment did not follow this trend and decreased over the season (Fig. 20). Overall, the main effects, treatment and date, and the interaction between the two, was significant ($P < 0.0001$).

Dormant bud numbers in the high-intensity treatment were significantly different from the control and low-intensity treatments for 8 months following treatment ($P < 0.05$). For these months, dormant bud numbers were lower for the high-intensity treatment than the other treatments.

Dormant bud numbers in the low-intensity treatment were different from the control for late November and February (respectively 4 months and 7 months following treatment) ($P < 0.05$). Dormant buds were higher for the low-intensity treatment than the control during these months.

The greatest dormant buds per tiller for the high-intensity treatment was in early June, and for both the low-intensity and control in February (Fig. 21). The average maximum dormant buds per tiller was 0.8±0.1 for the high-intensity treatment, 2.4±0.1 for the low-intensity treatment, and 2.8±0.1 for the control. The lowest dormant buds per tiller for the low-intensity and control treatments was in May with 0.3±0.1 and 0.4±0.1 buds per tiller respectively (Fig. 21). In the high-intensity treatment, the lowest dormant buds per tiller was 0±0.1 and first occurred in late September. There was a large difference in maximum dormant buds per tiller, and timing of this statistic, in the high-intensity treatment in comparison to both the low-intensity and control treatments.

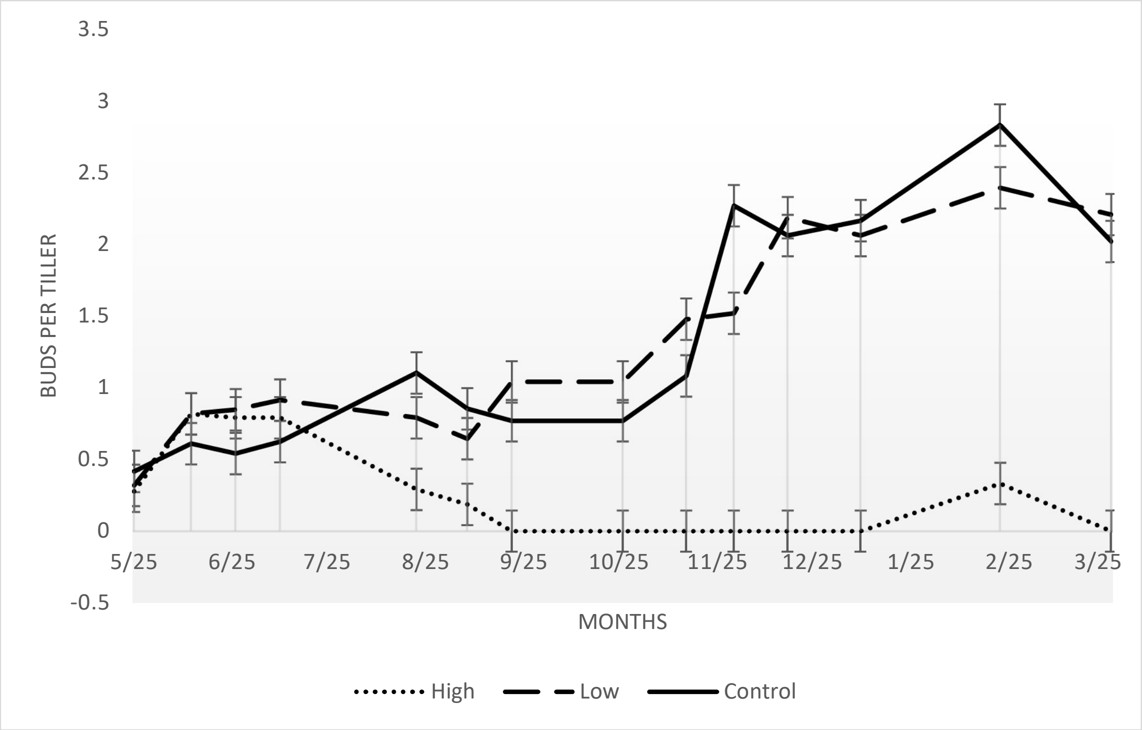

Figure 20: Dormant buds per tiller in the high-intensity, low-intensity, and control treatments over the year for *H. belangeri*.

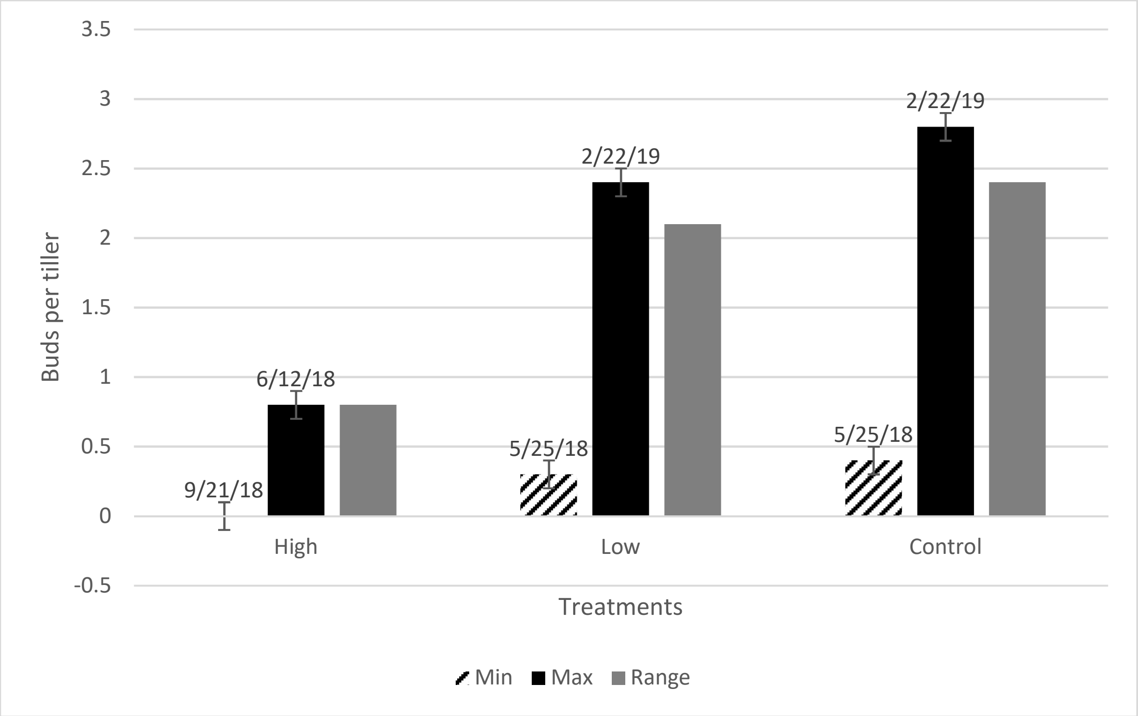

Figure 21: The minimum, maximum, and range of dormant buds per tiller for *H. belangeri*. Dates above bars indicate the date in which the max or minimum was reached.

Dead bud numbers, for the low-intensity and control treatments, were the highest in May (Fig. 22). However, for the high-intensity burn treatment, dead buds per tiller peaked in August (Fig. 22). This peak was significantly different from the other treatments ($P < 0.0001$). Despite these peaks, dead buds remained relatively low over the course of the season.

Overall, the main effects, treatment and date ($P = 0.0001$; $P < 0.0001$), and the interaction between the two ($P < 0.0001$), were significant. The only difference in dead bud numbers between the low-intensity and control treatments was during May, where the low-intensity treatment had a greater amount of dead buds as opposed to the control (*P* = 0.0258).

The maximum dead buds per tiller in the high-intensity treatment occurred in August, with 0.8±0.04 buds per tiller. The maximum dead buds per tiller in the low-intensity and control treatments occurred in May, with 0.20±0.04 and 0.10±0.04 respectively (Fig. 23). The minimum dead buds per tiller in all treatments was 0.00±0.04 and occurred multiple times over the season (Fig. 22; Fig. 23). Once again, the high-intensity treatment were different than the low-intensity and control treatments in regards to the maximum number of dead buds per tiller measured.

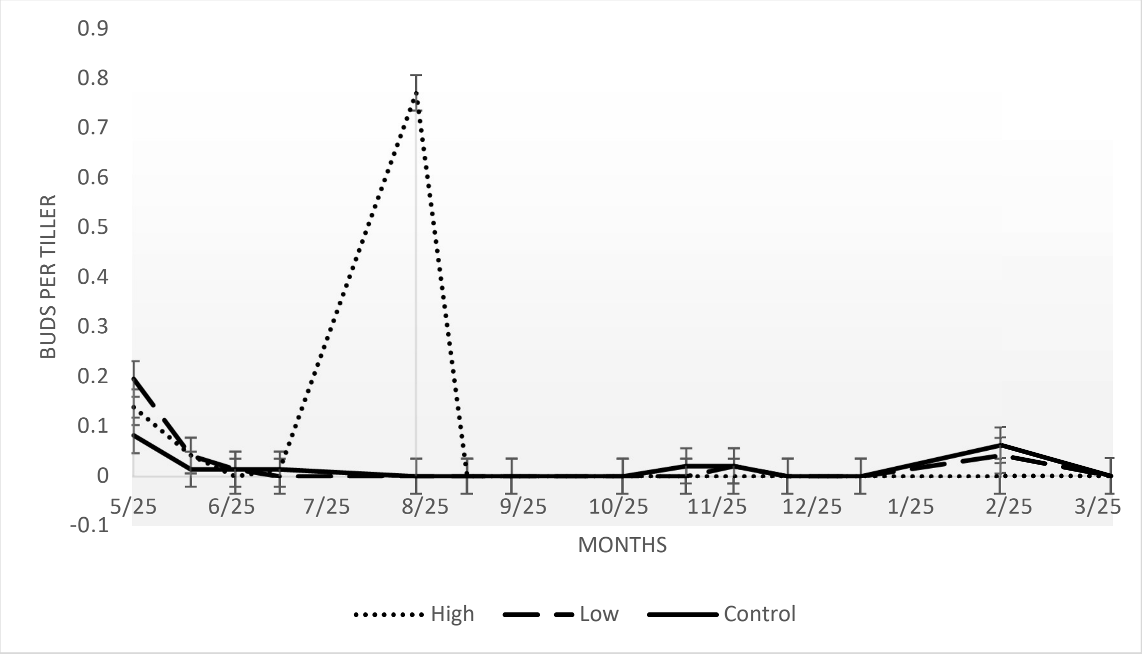

Figure 22: Dead buds per tiller in the high-intensity, low-intensity, and control treatments over the year for *H. belangeri*.

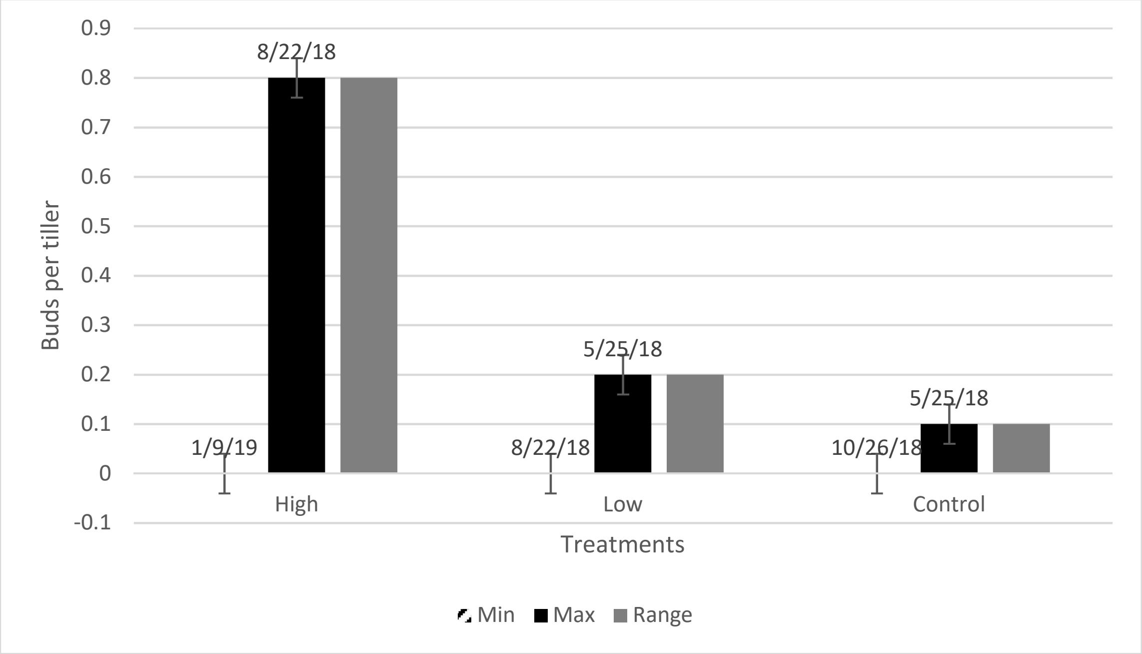

Figure 23: The minimum, maximum, and range of dead buds per tiller for *H. belangeri*. Dates above bars indicate the date in which the max or minimum was reached.

Discussion

Our results indicate that fire intensity differentially affected our graminoid species, with high-intensity fire treatments leading to direct mortality in both species but at different levels. For our C_3 species, *N. leucotricha*, the high-intensity treatment resulted in 0.33±0.04 dead buds per tiller in August. However, overall bud numbers and activity were equivalent in the high-intensity and control treatments by the end of our sampling period, eight months following treatment. On the other hand, our C_4 species, *H. belangeri*, the high-intensity treatment resulted in 0.77±0.04 dead buds per tiller in August. High-intensity treatment bud numbers and activity were significantly lower than the control with no indications of recovery 8 months post-treatment. These differing recovery rates in response to high-intensity fires may be a result of these species' contrasting photosynthetic pathways and growth forms.

In the control treatment, there was an apparent shift in abundance of dormant and active buds over the season for *H. belangeri*. Active buds peaked in May and decreased over time. At the same time, dormant buds were lowest in May, increased throughout the season, and peaked in February. This is typical of a C_4 species, in which the dormant bud bank is the largest going into winter dormancy (Dalgleish and Hartnett, 2006; Ott and Hartnett, 2012; Russell et al., 2015).

The low-intensity treatment followed the trends described in the control, with only a few significant differences. Total, active, and dormant bud numbers had small difference between the low-intensity and control treatments but, overall, followed similar trends. The only significant difference in dead buds per tiller between the low-intensity and control treatments was before treatment application. Overall, the impact of our low-intensity fires on *H. belangeri*'s bud bank did not cause our low-intensity treatment to deviate greatly from control plot trends over our 8 months of analyses.

On the other hand, the high-intensity treatment did not follow this trend. Following fire treatment, there was a sharp increase in dead buds. After this, dormant, active, and total buds decreased significantly until late September where no *H. belangeri* individuals created new tillers, producing zero buds. The high-intensity fires directly led to bud mortality and this may have impacted resprouting capability and subsequent recovery of individuals. Whether or not this lack of resprouting is simply a result of delayed emergence or full belowground mortality will require further data collection to assess.

There is strong evidence that C_4 grass species depend on large overwintering bud banks in order to survive the winter and to recruit new tillers at the beginning of the next growing season (Dalgleish and Hartnett, 2006; Ott and Hartnett, 2012; Russell et al., 2015). *H. belangeri* seems to be no exception. This stoloniferous grass may maintain a large population of buds in order to ensure rapid response to resource pulses, persist following disturbances, and ultimately to ensure population dominance. However, bud death may modify the size of the bud bank, impacting tiller recruitment during the following growing season, recovery following disturbance, and community dynamics. This data is important to consider because, if *H. belangeri* is incapable of recovering in areas in which *H. belangeri* is dominant or a favorable species, use of summer high-intensity fires to remove invasive woody shrubs may not be the most effective management decision.

In the control, total buds for *N. leucotricha* fluctuated largely through the season. *Nassella leucotricha* also had, overall, small accumulations of dormant buds. Fire treatments followed the same patterns over the season. Despite some significant differences between the fire treatments and the control for the few months after the burns, the total buds of the fire treatments were comparable to the control by February (7 months post-treatment). Although not statistically significant, the high-intensity treatment lagged behind in dormant buds until the final month of data collection, while the low-intensity treatment exceeded the control from January onwards. The only exception when it came to bud mortality was in August, where the high-intensity treatment experienced a significant increase in dead buds per tiller. These trends indicate direct bud mortality due to high-intensity fires but eventual recovery by the end of our data collection. All the while, the low-intensity fires trends may indicate increased productivity of *N. leucotricha* due to favorable post-fire conditions. This reveals that summer high-intensity fires can be used to remove invasive shrubs in areas in which *N. leucotricha* is a dominant and favorable species, without high repercussions for this graminoid species.

The direct mortality of *H. belangeri* buds and failed/delayed recovery in comparison to the quick and full recovery of *N. leucotricha* is most likely a result of growth forms and photosynthetic pathway. *Hilaria belangeri* is a stoloniferous grass. As such, the bud bank is very shallow and many buds are associated with stolons which have little to no protection from the soil. *Nassella leucotricha* is a bunchgrass, resulting in increased bud bank depth. Soil is a very good insulator so deeply buried buds would be better insulated from damaging heat (Valettel et al. 1994).

Phenology as a result of photosynthetic pathway most likely impacted bud bank dynamics due to the timing of our burns in late summer. Given that *H. belangeri* is a C_4 species, it was more active than *N. leucotricha*, a C_3 species, during our burns. C_3 species are dormant during the summer so fires during this time relate to C_3 functional group dominance (Steuter, 1987; Ewing and Engle, 1988; Russell et al., 2015). Therefore, it was predictable that our fires, including the high-intensity, did not cause irreparable damage to our C_3 species. On the other hand, winter and spring burns tend to stimulate C_4 species (Steuter, 1987; Ewing and Engle, 1988; Russell et al., 2015), so the increase in dead buds resulting from our fire treatments was understandably greater for our C_4 species than our C_3 species.

From the data collected, *N. leucotricha* recovered fully from our high-intensity treatment; however, *H. belangeri* seems to have experienced a significantly greater negative impact. These differences may be attributable to differences in photosynthetic pathway and growth form. Ultimately, these interspecific differences may explain and allow for predictions on how grasslands and savannas may respond to environmental changes and disturbances. In particular, these predictions will help determine grass recovery following high-intensity fires used as a management tool to remove invasive woody shrubs. Further data collection over the course of multiple growing seasons will be needed to fully evaluate the lasting impacts of these treatments on both grass species.

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CHAPTER IV

SUMMARY & CONCLUSIONS

In ecosystems dominated by perennial grasses, native graminoids have adapted to withstand frequent disturbances by resprouting from a belowground bud bank. In the presence of frequent disturbances, especially fire, regrowth from a bud bank that is insulated and protected by a layer of soil offers a competitive advantage. This form of vegetative reproduction is overwhelming prolific and allows grasses to not only recover from disturbances but also to take advantage of pulses in resource availability. Therefore, bud bank size and dynamics are a key component in grass recovery but also community structure of grass-dominated ecosystems.

With the removal of natural disturbances, there has been an increase in woody shrub invasions into grasslands and savannas. Once this woody encroachment has proceeded beyond a certain threshold, reintroducing fire back into the system is often not a viable means to return to a grass-dominated system. Despite this, previous studies have shown that the use of high-intensity fires during drought can result in mature woody shrub mortality. These studies largely studied the interaction between fire intensity and resprouting shrubs, but not much is known about graminoid response to high-intensity fires. Therefore, if high-intensity fires are to be a viable management tool for reversing woody encroachment, it’s imperative to understand how fire intensity interacts with graminoid bud banks.

This research investigated the impact of fire intensity on graminoid bud bank size and activity. The study was conducted in a semiarid savanna system in the Edwards plateau ecoregion of Texas. We chose two abundant and dominant grass species, *Nassella leucotricha* and *Hilaria belangeri*, with contrasting growth forms and photosynthetic pathways, to be the focus of our research. Our treatments consisted of 12 no burn (control) plots, 12 low-intensity fire plots, and 12 high-intensity fire plots.

We found that fire intensity had a significant impact on the number of total buds, the number of dead buds, and the number of active buds within 24hr following our treatments. For both of our species, high-intensity fires led to an immediate decrease in the number of total and active buds and increased the number of dead buds. However, high intensity fires led to a greater increase in dead bud numbers in *H. belangeri* than in *N. leucotricha* immediately following our burns. However, despite significant bud mortality, *N. leucotricha* individuals resprouted three weeks following high-intensity fires. This could not be said for *H. belangeri* where only 25% of individuals resprouted three weeks following treatment. Over the long-term, bud numbers and activity in the high-intensity treatment were equivalent to those in the control 8 months following treatment for *N. leucotricha*. However, *H. belangeri* bud numbers and activity were significantly lower in the high-intensity treatment when compared to the control, even after 8 months. These differences between species and fire intensity treatments was not a result of induced soil hydrophobicity. Instead, they were likely a result of differences in bud depth, resulting from differential growth forms, and phenology dictated by photosynthetic pathway.

Average bud depth was greater for *N. leucotricha* than *H. belangeri*. This difference is likely a result of growth forms, with *N. leucotricha* being a caespitose grass and *H. belangeri* being a stoloniferous grass. Bud depth is important to note because soil is an effective insulator, so buds deeper in the soil profile will likely be more protected from heat exposure. Similarly, photosynthetic pathway may have an influence on the impact of fire intensity on bud bank dynamics due to temporal variations in bud activity. Our fires were set in the summer, in which our C_4 grass, *H. belangeri*, was most likely active. On the other hand, our C_3 grass, *N. leucotricha*, was likely dormant during this time. Since actively growing tissues are more easily damaged by fire than dormant tissues, *N. leucotricha* individuals were likely less affected by our high intensity fires than *H. belangeri*.

To fully evaluate long-term trends, more research is needed, especially to evaluate whether the lack of resprouting by *H. belangeri* was due to full belowground death or induced early dormancy. There is also evidence that full recovery following fire may not be seen until the 2nd growing season. More research may also be needed in order to evaluate any legacy effects from our burns. There is also a necessity to understand how high-intensity fires in semiarid savanna systems impact soil nutrients and soil microorganisms. Edaphic conditions following fire can have a large impact on plant community dynamics and probably underground bud bank dynamics.

The goal for this research is to understand how fire intensity affects graminoid bud banks in order to advise on the survival of grasses following high-intensity fires. This research will also serve to formulate management recommendations based on differences in species ecophysiology such as growth forms and photosynthetic pathway. This method of land management may allow landowners to restore their rangelands and increase the overall health of grass dominated ecosystems in areas in which human alteration has led to invasion by woody plants.