GRAZING AND DROUGHT IN TALLGRASS PRAIRIE: THE ROLE OF BELOWGROUND BUD BANKS IN VEGETATION DYNAMICS

by

BENJAMIN LEE VANDERWEIDE

B.S., Calvin College, 2007

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the
requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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KANSAS STATE UNIVERSITY
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ABSTRACT

Grazing and drought are instrumental in the development and maintenance of perennial grasslands. In this research I tested the belowground bud bank contribution to tallgrass prairie resistance and resilience when perturbed by grazing and drought. First, I tested the bud bank role in vegetation response to and recovery from severe drought (Chapter 2). I compared above- and belowground responses of experimentally droughted plots to ambient controls and irrigated plots during two years of severe drought and two years of recovery. I found that although aboveground net primary productivity declined 30-60% during drought, bud bank density and demography were insensitive to drought. These results suggest that grassland resistance and resilience when perturbed by drought may be mediated by stability of belowground bud banks. Second, I investigated vegetation and soil nutrient legacies following release from long-term grazing (Chapter 3). I documented a relatively rapid shift in aboveground vegetation within four years of grazer exclusion, with productivity, stem density, and diversity becoming relatively more similar to ungrazed than grazed prairie. The density and composition of the belowground bud bank and soil seed bank shifted more slowly, remaining more similar to grazed than ungrazed prairie. Responses of soil nutrients to removal of grazers varied, and in some cases was affected by recent fire history. These results demonstrate the contribution of belowground propagules to the maintenance of a diverse plant community both during grazing and after grazers are removed. Finally, I examined short-term vegetation responses to both drought and grazing (Chapter 4). Despite extreme drought and simulated grazing that reduced productivity and increased mortality of individual stems, the dominant C_4 grasses maintained a stable bud bank. Aboveground net primary productivity and bud bank density of sedges and forbs, however, were reduced by both drought and grazing. This differential response of species to extreme drought and grazing led to shifts in community composition and species diversity over one growing season. Across drought and grazing treatments, live rhizome biomass was highly correlated with bud bank density and may be a useful, more easily measured index of bud bank density.

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Dedication

I dedicate this dissertation to two people, the first who initially sparked my interest in studying plant ecology, and the second who encouraged and supported me through the process of earning my Ph.D.

I dedicate this dissertation to David Warners. His botanical knowledge, mind for discovery, and heart for serving others continue to be a model for me.

I dedicate this dissertation to my wife, Debbie. Through thick and thin, she encourages me to pursue my dreams.

Chapter 1

The role of bud banks in the vegetation dynamics of tallgrass prairie

1.1 Definition and Description of the Bud Bank

Although plants are sessile organisms, the undifferentiated cells of meristems allow them to respond to changing conditions and obtain resources from a heterogeneous environment. Plants alter the birth and death rates of the modules that compose them in response to resource availability in the environment. Buds play an important role in the demography of plant parts. Buds are embryonic shoots that contain a meristem protected by young leaves. Environmental factors such as temperature, nutrient concentrations, water availability, and light quality or quantity control bud outgrowth (Anderson et al. 2001, Williamson et al. 2012). The relative sensitivity of the buds of different plant species to these cues may determine the tolerance of a species to different disturbance regimes (Lehtila 2000). Buds provide a reservoir of meristems that can be initiated after injury from herbivory, fire, drought, or severe weather events, or seasonally when favorable growth conditions resume after winter or the dry season (Klimešová and Klimeš 2007). In his seminal work on plant population biology, John Harper coined the term *bud bank* to describe this reservoir of buds (Harper 1977).

I established a research program to test the effects of drought and grazing on the vegetation dynamics of tallgrass prairie and to determine the role of the bud bank in those dynamics. This research seeks to test and extend the results of earlier studies on bud bank dynamics in tallgrass prairie. Those studies clearly established that the belowground bud bank is the source of nearly all aboveground stems in tallgrass prairie (Benson and Hartnett 2006). Even when disturbed by pocket gophers (*Geomys bursarius*), the majority of the regrowth is from belowground meristems, not from seed (Rogers and Hartnett 2001). Dalgleish and Hartnett (2006) demonstrated that the availability of meristems may constrain the ability of grasslands to adjust aboveground net primary productivity in response to precipitation, supporting the meristem limitation hypothesis (Knapp et al. 2001). Further studies in this system demonstrated that total bud bank density is greatest and forb abundance lowest in annually burned, ungrazed areas, while fire exclusion and grazing are associated with lower total bud bank density and greater forb abundance in the bud bank (Benson et al. 2004, Dalgleish and Hartnett 2009).

Detailed studies of the bud bank dynamics of individual species helped explain plant responses to fire, grazing, and nutrient availability in tallgrass prairie. Ott and Hartnett (2011) found that flowering tillers of *Andropogon gerardii* Vitman, a dominant C_4 grass in tallgrass prairie, produced more buds than vegetative tillers, and these buds grew out into stems at a higher rate than buds on vegetative tillers. Ott and Hartnett (2012) also described the phenology and demography of the bud bank for *A. gerardii* and a C_3 grass, *Dichanthelium oligosanthes* (Schult.) Gould. *Andropogon gerardii* produced nearly all buds each year quickly at the beginning of the growing season, and the bud bank was composed of buds of multiple age classes, up to 3 years old. In contrast, *D. oligosanthes* buds lived less than one year, and while some buds were produced in the spring, most buds were produced at the end of the growing season. N'Guessan and Hartnett (2011) found that while productivity and relative growth rates of *Schizachyrium scoparium* (Michx.) Nash were reduced by grazing, bud production per tiller was constant across a range of clipping intensities. Dalgleish et al. (2008) demonstrated that nitrogen availability affects bud bank demography for both a C_3 and a C_4 bunchgrass. Finally, a growth chamber study by Williamson et al. (2012) found that while C_3 grasses responded to light and nitrogen as predicted by the model of Tomlinson and O'Connor (2004), but C_4 grasses did not respond as predicted, suggesting that a different model may be needed to explain bud activation in C_4 grasses.

These studies clearly demonstrate that most aboveground stems in this system are derived from belowground buds, and that the density and composition of the bud bank varies with fire, grazing, and precipitation. However, it is not clear if bud bank density and demography drive plant population and community dynamics, or if bud density and demography only reflect changes in plant growth and reproduction. To introduce my research program, I first present a conceptual model describing the dynamics of a tallgrass prairie bud bank in the context of overall perennial plant growth. I then introduce the individual research projects I completed to test the effects of drought and grazing on bud banks and vegetation dynamics in tallgrass prairie.

1.2 Perennial Plant Growth and Bud Banks Dynamics

In tallgrass prairie the buds associated with perennial belowground stems (*e.g.* rhizomes, bulbs, corms, and tubers) are responsible for persistence of perennial plant species from year to year, while aboveground stems and their associated buds are replaced annually. Axillary buds are borne on phytomers, the basic plant module that includes the internode and node of the stem, the leaf, and the axillary bud in the axil of the leaf. The production of axillary buds is intimately connected with the addition of new phytomers to rhizomes and other belowground stems, and the longevity of buds is necessarily less than or equal to the longevity of the parent phytomer (Harper 1977). Therefore, processes and events that affect plant growth and survival also affect the production and survival of buds.

Bud development begins with the growth of the main stem axis and the addition of new phytomers. Axillary or accessory buds are formed in the axils of leaves, and the apical or terminal bud is located at the apex of the stem. Buds begin as primordia in meristems and grow to produce an embryonic stem covered with embryonic leaves. The most basal embryonic leaves often develop into a protective prophyll that encloses the bud during its dormant stage. Generally one bud is produced per phytomer, but there are exceptions. Plants with opposite branching produce two buds per phytomer, while grasses and sedges may bear buds on nodes at the culm base, but no buds at nodes on the “neck” of the rhizome (Liese 1998). Buds are associated with both aboveground stems and belowground stems. Belowground stems are often modified to perform various functions (e.g. rhizomes allow lateral dispersal, while corms and tubers store resources), but all retain buds that allow growth of new stems.

Unlike seeds, buds are physiologically connected to and dependent on the parent plant; they generally do not store large quantities of nutrients or disperse from the parent plant except through the growth of the bud-bearing stem or plant fragmentation due to damage. Because young shoots from buds are larger and grow more quickly than shoots that originate from seeds, it can be advantageous for a plant to maintain a reserve of dormant buds for regrowth after the dormant season or after injury (Harper 1977, Landa et al. 1992). While competition with the parent ramet may result from limited dispersal, competitive exclusion of heterospecific plants and resource sharing through physiological integration may provide a net benefit to a clone. Vegetative expansion of clones may therefore allow plants to more fully use favorable microsites.

Development of perennial plants of tallgrass prairie throughout the year can be divided into phases, as shown in Figure 1.1. After the dormant season, increased temperature, soil moisture, and light cue belowground buds to grow into aboveground stems (Williamson et al. 2012, Knapp and Hulbert 1986, Hulbert 1969). During this period plants are rapidly adding new phytomers and associated buds belowground as rhizomes elongate (Ott and Hartnett 2012). After initiating new stems, plants invest energy into development of leaves aboveground to increase photosynthetic capacity. With development of the leaf canopy, plants capture and store energy belowground in roots and rhizomes (or other stems). Finally, as temperatures cool in the fall and days shorten, plants translocate energy and nutrients from aboveground stems to belowground organs. Stems then die back to the soil surface as winter approaches. This model assumes that stem mortality is 100% each year, although many *Sorghastrum nutans* L. Nash stems survive for two years (McKendrick et al. 1975).

Grazing and drought may affect the plant community by altering stem demography, energy capture/storage, and/or senescence of aboveground stems (Figs. 1.1 and 1.2). Grazing removes aboveground biomass, allowing light penetration to the soil surface, increasing soil temperature, and triggering growth of new stems from buds (Hulbert 1969). If grazing continues through the growing season, stems continue to be recruited from the belowground bud bank, but energy capture and storage may be limited because plants must continually regrow aboveground (Fig. 1.2b). Since most stems are initiated early in the growing season while moisture is not limiting, drought may have little effect on production of new stems or associated buds (Fig. 1.2c). However, drought may prevent development of the plant canopy and limit energy capture and storage, and severe drought may trigger early senescence of aboveground stems. Since bud production is linked to growth of new stem units (phytomers), disturbances that affect stem initiation and growth may have the greatest effect on the bud bank. Mortality of belowground stems and associated buds in response to drought and grazing may be caused by age, depletion of stored energy, desiccation, or attack by belowground herbivores.

1.3 Investigations of the Effects of Drought and Grazing on Prairie Vegetation

I established a research program to test the effects of drought and grazing on the vegetation dynamics of tallgrass prairie, and the role of the bud bank in those dynamics. I first tested the role of the bud bank in plant community response to and recovery from drought (Chapter 2). I hypothesized that the belowground reserve of buds associated with rhizomes and other perennating organs would provide a stable source of recruitment to aboveground stems following short-term, severe drought. Detailed studies by John E. Weaver and colleagues documented widespread mortality of perennial plants due to long-term, severe drought across the Great Plains of North America in the 1930s (Weaver and Albertson 1936, Albertson and Weaver 1942). Bud banks will not be able to buffer population dynamics under such extreme circumstances when prolonged drought causes mortality, but may be very important in the more frequent, short-term droughts of this region. I experimentally reduced precipitation 76% from ambient levels for 2 years, and compared responses to ambient control and irrigated plots during the 2 years of drought and 2 years of recovery.

Next, I investigated the role of bud and seed banks in tallgrass prairie recovery from long-term grazing (Chapter 3). Belowground buds of clonal plants are comparable to seeds because both have the potential to produce physiologically independent plants. However, the relative importance of the seed and the bud banks varies depending on the type and severity of disturbance. Furthermore, genetic variability may be higher if genetically distinct individuals are recruited from seed compared to genetically identical stems that arise from belowground buds. In annually burned tallgrass prairie, less than 1% of established stems at the end of the growing season arise from seed (Benson and Hartnett 2006). Plants newly established from seed often develop a bud bank quickly so that their growth is not meristem limited (Klimešová and de Bello 2009). Similarly, Latzel et al. (2008) found that in urban plant communities, unless all of the existing vegetation is killed, the majority of the recruitment after disturbance is from the bud bank. In boreal forest, most recruitment of new growth after severe fires is from the seed bank, while after mild fires most plant resprout from the bud bank (Lee 2004). Because both buds and seeds may persist for many years in the soil, they many buffer plant populations and allow storage effects. I tested the hypothesis that soil seed and bud banks facilitate persistent legacies of grazing in tallgrass prairie.

Finally, I crossed drought and grazing in a factorial treatment design to determine if drought and grazing cause more damage together than when they are applied separately (Chapter 4). Previous studies suggest that a single season of grazing under drought conditions may have little effect on the plant community, but grazing in subsequent years may cause rapid shifts in plant community composition toward more ruderal or grazing-tolerant species (Weaver and Hougen 1939). Drought may limit resprouting after herbivory, preventing plants from storing energy for future growth. I document both above- and below-ground vegetation responses to these treatments through one growing season of drought and simulated grazing. The natural drought of 2012, combined with the experimental drought, provided a rare opportunity to study the response of bud bank dynamics to extreme drought conditions.

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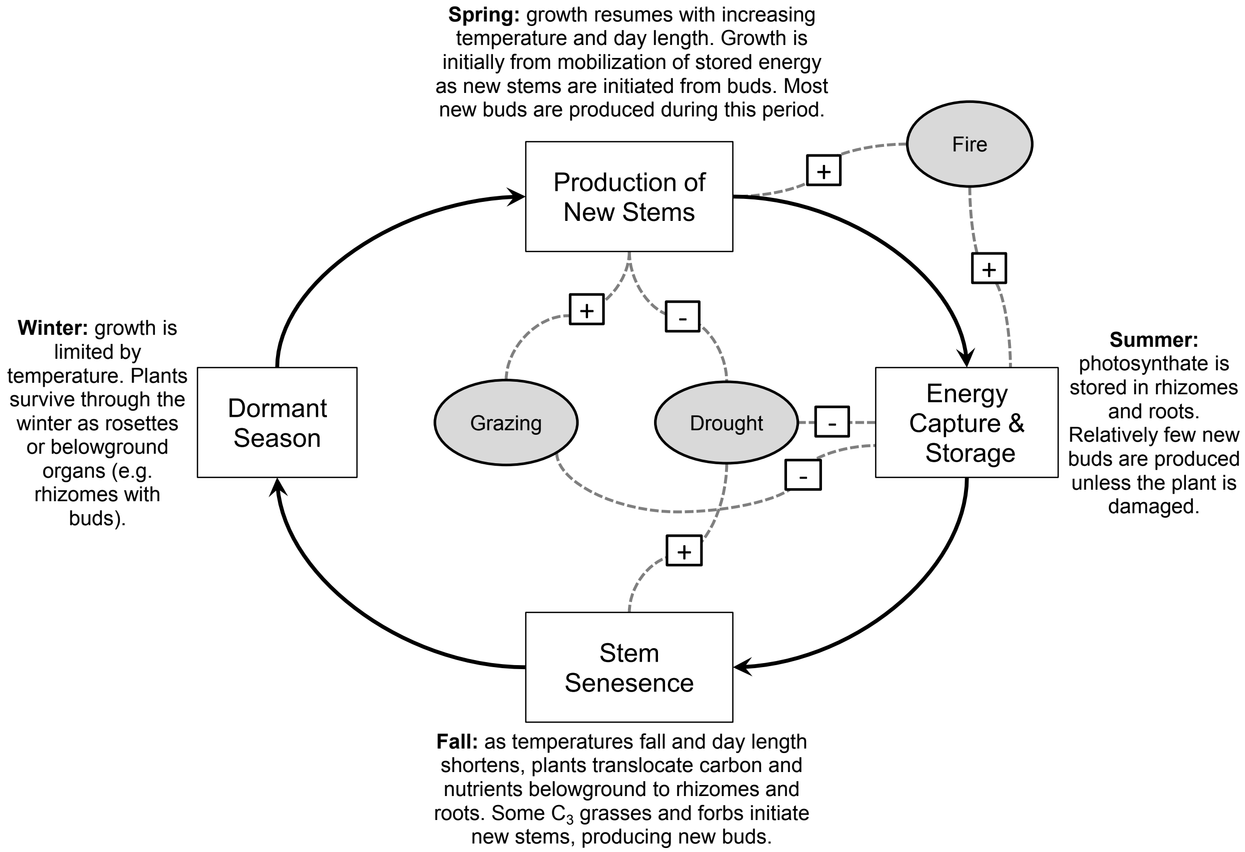

Figure 1.1: Conceptual diagram illustrating the effect of grazing, drought, and fire on perennial plant growth in tallgrass prairie. Boxes represent phases in the annual cycle of plant growth. Gray ovals indicate key disturbances, and the dashed gray lines connecting ovals to boxes indicate whether a disturbance enhances (+) or inhibits (−) that phase. Plant growth, and particularly the production of new phytomers, control the production of axillary buds.

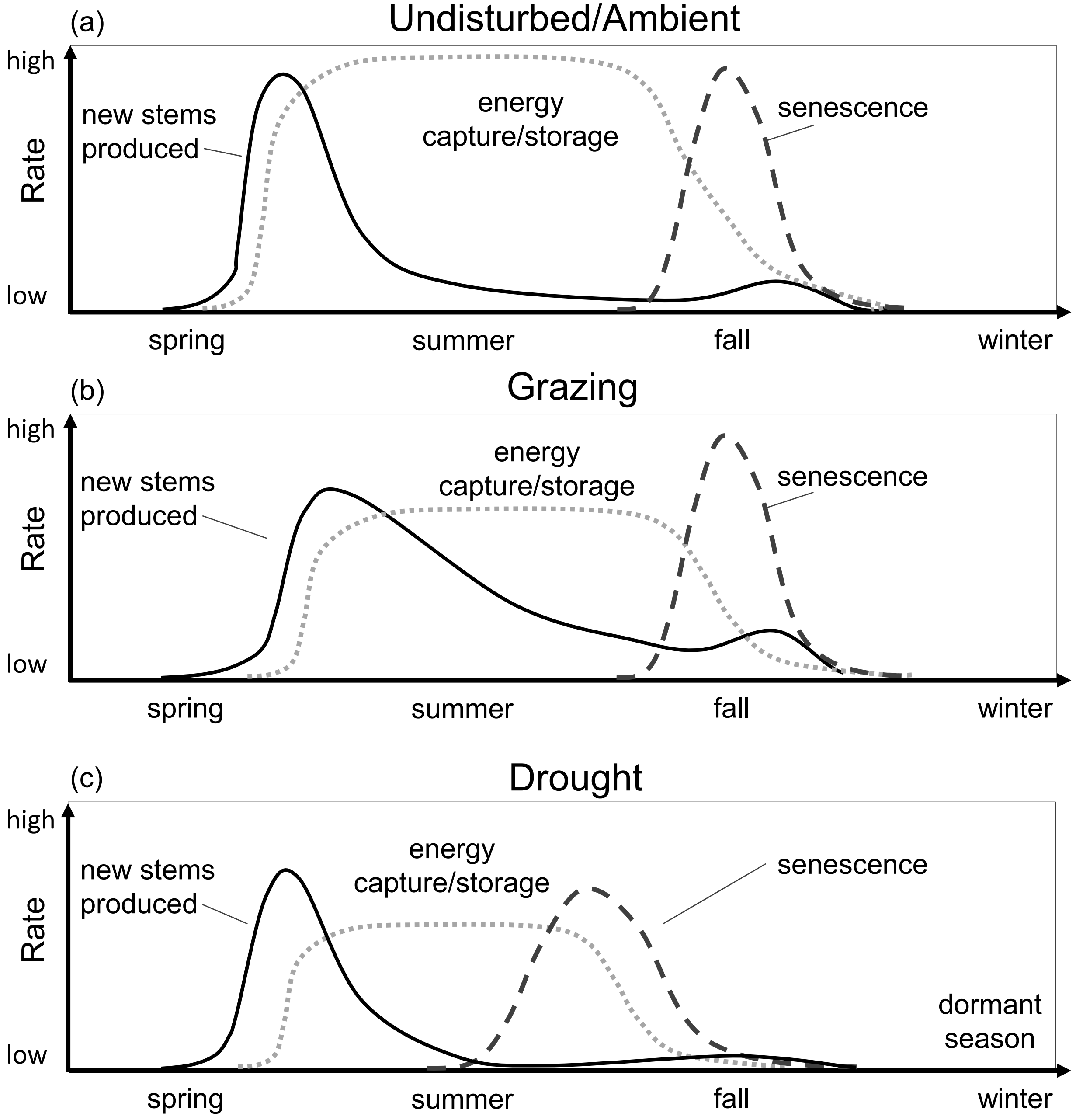

Figure 1.2: Compared to undisturbed prairie (a), grazing (b) promotes initiation of new stems, but removal of aboveground biomass limits energy capture and storage. Drought (c) may not affect production of new stems, but may limit energy capture and storage and cause early senescence of aboveground stems. The *y*-axis indicates the relative rate of stem initiation (solid black line), energy capture/storage (dotted gray line), and stem senesence (dark gray dashed line).

Chapter 2

Bud bank stability mediates grassland resilience following drought

Abstract

The belowground bud bank in tallgrass prairie may mediate plant community responses to and recovery from disturbance, including persistent drought. We hypothesize that the belowground reserve of meristems associated with rhizomes and other perennating organs provides a stable source of aboveground stems following impacts of severe drought. To study the role of bud banks in plant community response to persistent drought, we experimentally reduced precipitation 76% from ambient levels for 2 years and compared responses to ambient control and irrigated plots during the 2 years of drought and 2 years of recovery. We measured belowground bud bank density and aboveground stem density, canopy cover, and productivity. During this severe experimental drought, we observed reductions in aboveground productivity and C_4 grass flowering stem density. When favorable conditions returned, productivity and flowering stem density did not differ from ambient controls. In contrast, we observed no differences in bud bank density and stem density between drought plots and ambient controls in any year. The bud bank density and stem density of irrigation plots, however, were greater than both drought and ambient control plots during two years of recovery. This suggests that resistance and resilience of tallgrass prairie vegetation in response to short-term severe drought is primarily due to the stability of the belowground population of reserve meristems. The majority of bud outgrowth into aboveground stems, and resulting production of new buds, occurs early in the growing season before peak water stress is realized. Therefore bud

and stem densities may be relatively insensitive to water stress later in the growing season. Continued growth and reproductive effort, however, are dependent on water availability, resulting in the strong sensitivity of these responses to drought.

2.1 Introduction

Grass-dominated ecosystems cover over one third of the global land surface, provide essential ecosystem services, and support a diverse biota (Curtin and Western 2008). Climate, including periodic drought, has played a key role in the development and maintenance of grasslands (Anderson 2006, Beerling and Osborne 2006, Stromberg 2011). However, rapid climate change is expected to alter historical precipitation patterns, leading to increased temporal variability in precipitation, more severe drought, and reduced soil moisture (IPCC 2007, Jung et al. 2010, Min et al. 2011). To predict plant community responses to this change, we must understand plant traits that influence community stability.

The effects of water stress on individual grassland plants are well studied, but focus primarily on aboveground responses and traits (e.g. Knapp 1985, Ocheltree et al. 2012, Tucker et al. 2011). It is not well known if the drought tolerance of plants studied individually, and often *ex situ*, can predict the strength and sign of interactions within a plant community. Furthermore, belowground traits, including characteristics of roots and perennating organs, also play an important role in drought tolerance (Hartnett et al. 2013), but are rarely studied. In herbaceous plant communities, these belowground organs contain energy reserves and buds that allow survival and recovery when aboveground biomass is damaged or destroyed by disturbance. Therefore, belowground plant traits may be particularly important in grassland response to and recovery from disturbance, including drought.

In ungrazed, annually-burned tallgrass prairie of central North America, nearly all aboveground stems are recruited from the belowground buds associated with rhizomes and other perennating organs [“bud bank” *sensu* Harper (1977)] while less than 1% of established aboveground stems originate from seeds (Benson and Hartnett 2006). Vegetative growth can also account for the majority of recolonizing stems at various scales of soil disturbance (Rogers and Hartnett 2001), and are important for plant community response to drought in a newly established tallgrass prairie restoration (Carter et al. 2012). Because recruitment from seed is rare, the effect of drought on the presence and abundance of species in a plant community may be determined by its effect on the growth of buds into stems and the production of new buds by stems. Until recently few studies had examined the role of bud banks in population or community dynamics (Klimešová et al. 2011*a*, Ott and Hartnett 2011), or in plant community response to and recovery from disturbance (Carter et al. 2012, Klimešová et al. 2008, 2011*b*, Latzel et al. 2008).

Stability of the plant community may be mediated through bud bank demography. Resistance, the ability of the community to avoid displacement when perturbed, may be enhanced by rhizomes and associated buds that live for two or more years (Ott and Hartnett 2012, Hendrickson and Briske 1997). These long-lived propagules may also increase resilience, the rate at which the community returns to its former state after disturbance. Previous work suggests that bud bank dynamics may control the resistance and resilience of plant productivity when perturbed by drought. Interannual variation in grassland productivity increases as bud bank density increases (Benson and Hartnett 2006, Dalgleish and Hartnett 2006), suggesting that the availability of meristems may constrain the ability of plants to respond to water availability (Knapp and Smith 2001). The belowground bud bank may stabilize plant community composition during drought, allowing productivity to respond dynamically to water availability when favorable conditions return. Thus aboveground resilience following drought may driven by belowground resistance to drought.

The overall goal of this study was to assess the role of bud bank demography in plant community response to and recovery from severe drought. We[1] were guided by three objectives: (1) determine the sensitivity of the transition from bud to stem and the number of buds per stem to drought, (2) assess the contribution of the bud bank to stability of plant productivity, and (3) compare the resistance and resilience of plant community composition above- and belowground.

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[1] The vegetation data from this chapter was collected in collaboration with Daniel Carter. DC collected stem density and canopy cover data in 0.25 m^2 quadrats. BV designed the study, collected all other data, designed analyses, performed analyses, and wrote the manuscript. All authors discussed the results and commented on the manuscript. To reflect this collaboration, I use “we” in this chapter.

2.2 Materials and Methods

2.2.1 Site Description

Konza Prairie Biological Station (KPBS) is a 3487 ha tallgrass prairie preserve in the Flint Hills of northeast Kansas, USA (39°05′ N, 96°35′ W). KPBS experiences a continental climate, with warm, wet summers and cool, dry winters. Mean monthly air temperatures range from -2.7 °C in January to 26.6 °C in July. Total annual precipitation averages 880 mm [1983-2012, NOAA National Climate Data Center (2013)], with 79% falling during the growing season (April to October). However, precipitation patterns in this region are highly variable among years, ranging from 513 to 1435 mm annually in the period 1983-2012. A matrix of C_4 grasses dominates the vegetation, including big bluestem (*Andropogon gerardii* Vit.), Indian grass (*Sorghastrum nutans* [L.] Nash), and little bluestem (*Schizachyrium scoparium* Michx.), with a wide variety of subdominant C_4 grasses, C_3 grasses, and forbs interspersed (Towne 2002).

All study plots were located on level upland native (unplowed) tallgrass prairie, with shallow upland Florence cherty silt loam soil (Soil Survey Staff 2012). The study area was not grazed during the 1970s and 1980s, was lightly grazed by cattle (*Bos taurus*) at 6.1 ha per animal unit in 1992 and 1993, and has not been grazed since. The study area was burned on average every two years until 1994, then burned annually in March or April from 1994 until 2012 (but not burned in 2010). Twelve total 5 by 5 m study plots were blocked by location within the watershed in a randomized complete block design. Three precipitation treatments (severe drought, irrigated to prevent water stress, and ambient conditions) were randomly assigned to plots within four blocks.

2.2.2 Precipitation Treatments

We used passive rainfall interception structures to exclude 76% of the incident precipitation from mid-March to October of 2009 and 2010 using a modification of the shelters tested by Yahdjian and Sala (2002). Shelters were 4.5 by 4.5 m with two rows of 2.4 m by 0.2 m clear Lexan® shingles (SABIC Innovative Plastics, Pittsfield, Massachusetts, USA) sloping to the north and south at 15° from the central peak. This orientation of the shelters takes into account prevailing southerly winds in the summer. Shingles were bent lengthwise at 120° and spaced to cover 80%

of the total area covered by the shelter (Figure 2.1). Using a portable spectrometer (Analytical Spectral Devices (ASD) FieldSpec Pro, Boulder, Colorado, USA), we determined that at least 90% of visible light was transmitted through the shingles (Figure 2.2). True rainfall interception was 76% of ambient precipitation, as determined using paired rain gauges underneath and outside each shelter. All water collected by the shingles was drained at least 2 m away from the shelter on the downhill side. In September 2010 point measurements of gravimetric soil moisture in the top 10 cm revealed a 10% decline in soil moisture in the central 3 by 3 m area of drought plots relative to ambient plots. Drought plots were irrigated in June, July, and August of 2011 due to unusually low precipitation according to the protocol described below used for the irrigated plots.

Plots assigned to the irrigation treatment were irrigated from May to September in 2009 and 2010 to satisfy estimated evapotranspiration (ET) using the general methods of the KPBS long-term irrigation transects (Knapp et al. 2001). The goal of irrigation was to maintain volumetric soil moisture near or above 0.25 cm^3/cm^3. Reference ET was calculated to the FAO-56 standard using the Penman-Monteith equation (Allen 2000), and precipitation was measured using a rain gauge within 1 km of the plots. Annual precipitation recorded at Konza Prairie was average or above average for 2008, 2009, and 2010 (1153 mm, 987 mm, and 842 mm, respectively) but below average for 2011, and 2012 (815 mm and 570 mm, respectively), with 82, 84, 85, 64, and 74% falling during the growing season each year from 2008 to 2012 (Konza HQ1MET weather station). Irrigation was generally applied in the morning using a hand-held spray nozzle at 13 liters min^{-1}. Rainfall equivalents of 16 to 30 mm were applied to each plot during an irrigation session, depending on soil moisture deficits and forecasted weather. In total, the equivalent of 86 mm of additional water was added to irrigated plots in 2009 and 125 mm in 2010 (+16% and +20% of April to October precipitation, respectively). Due to extremely dry conditions in 2011, water was applied to the drought and irrigated plots to ensure post-drought recovery (122 mm, 27% of April to October precipitation). No precipitation treatments were applied in 2012. Rainfall was not manipulated in any way on ambient control plots from 2009-2012.

2.2.3 Plant Sampling

To avoid edge effects, including horizontal movement of water through the soil and resource sharing via rhizomes of clonal plants, all samples were taken from the central 3.5 by 3.5 m area of each plot. To estimate bud bank density (2008 to 2012), we harvested 10 by 10 cm belowground samples to a depth of 10 cm at random locations within plots. Depth of 10 cm was chose because previous work found that the mean depth of grass and forb rhizomes 3.2 ± 2.1 cm (mean pm SE), with few rhizomes below 12 cm (Elder 2001). Bud bank sampling occurred in the dormant season (November to February) to avoid fluctuations in density that result from stem recruitment from the bud bank during the growing season (Dalgleish and Hartnett 2006). Ten belowground samples per plot were extracted each year except 2008, when only eight were collected. Because destructive soil core sampling limits long-term sampling within the plots, 2008 pre-treatment belowground samples were extracted from random locations within 2 m of each plot. Samples were placed in plastic bags immediately after extraction and stored at 4 °C until further processing. Processing involved rinsing soil from the belowground samples (no more than 3 weeks prior to examination) and examining belowground structures with a dissecting microscope, trimming roots as necessary to allow careful observation. Belowground buds were counted and assigned to graminoid or forb (2008) or to species (2009 to 2012) when possible using bud and rhizome morphology, phyllotaxy, morphology of the attached root system, and morphology of any remaining aboveground parts, as in Carter et al. (2012). Because many forbs could not be confidently assigned to species using belowground morphology and were relatively rare, they were pooled for all bud bank analyses.

Additional measurements were taken in 2012 to estimate different fractions of belowground biomass in the top 10 cm of soil. Fractions included total root mass, live rhizome mass by species, and total dead rhizome mass. All fractions were dried at 60 °C for $\geq$ 48 h and weighed to the nearest 0.01 g.

Peak stem density was estimated in two ways. First, stems were counted to species in four systematically located 0.25 m^2 quadrats per plot in both May and August, with peak stem densities of each species used for analysis (2009 to 2012). Second, before excavation all stems directly above the 10 by 10 cm belowground samples were counted in September and assigned to graminoid or forb (2008 to 2012). Flowering stems of C_4 grasses were counted within the four quadrats in September (2009 to 2012). Peak cover of all species was estimated in the four quadrats per plot using a modified Daubenmire scale (Daubenmire 1968) in both May and August. Aboveground net primary productivity (ANPP) was estimated by harvesting peak standing biomass with scissors at the soil surface in late August to mid-September within four (2009) or five (2010 to 2012) randomly located 0.125 m^2 quadrats per plot. Biomass was dried at 60 °C for $\geq$ 48 h and weighed to the nearest 0.01 g.

2.2.4 Statistical Analyses

All statistical analyses were performed with R 3.0 (R Core Team 2013). Subsample measurements within plots were combined by calculating the mean of each response within each year. The assumption of normality of response variables was checked by visual inspection of normal quantile (Q-Q) plots and using the Shapiro-Wilk test. Responses of aboveground biomass, C_4 flowering stem density, bud density, stem density, buds per stem, and stems per bud were compared among treatments and years with mixed model ANOVA using the *nlme* package (Pinheiro et al. 2013). The number of buds per stem, a measure often used as an index of meristem limitation in perennial grasses (Dalgleish and Hartnett 2006), was calculated by dividing the mean bud density of the ten 0.01 m^2 soil cores within each plot in year t by the mean stem density directly above those soil cores in the same year. Stems per bud, a measure of bud growth into aboveground stems, was calculated by dividing the stem density in year $(t + 1)$ by the bud density in year t. Precipitation treatment, year, and the interaction between treatment and year were modeled as fixed effects. Year was a repeated measure, so I determined the most appropriate correlation structure by comparing the Akaike Information Criterion (AIC) for models fit with different structures [no correlation structure, compound symmetry, unstructured, and AR(1)]. The model that minimized AIC was used for analysis. Block was modeled as a random effect. Model fit was tested through visual inspection of QQ plots and by plotting fitted values against standardized residuals. Where heteroscedasticity was evident, I specified a power covariance structure with the fitted model values. I performed *post hoc* contrasts to identify drivers of significant main effects and interactions, using the *phia* package in R. Although not an appropriate sub-hypothesis of the interaction test, pairwise comparisons of treatments within years with Bonferonni-adjusted p-values are also reported because they are commonly used in ecology. All grass-like plants (grasses, sedges, and rushes) are referred to as "graminoids," while non-graminoid herbaceous plants are referred to as "forbs."

Measures of Resistance and Resilience

We calculated resistance to precipitation treatments and resilience during the recovery period for aboveground biomass (ANPP), C_4 grass flowering stem density, total stem density, and total bud density using absolute change of response in treatment plots (irrigated or droughted) relative to ambient plots within each year (van Ruijven and Berendse 2010). We define resistance as response to a precipitation treatment during the years of the treatment (2009 and 2010) relative to the ambient control. We define resilience as response to a precipitation treatment in years following precipitation manipulation (2011 and 2012) relative to the ambient control. Absolute change in a response was calculated using the difference between responses observed in irrigated or drought plots versus ambient plots.

Analysis of Community Composition

We evaluated shifts in community composition across years in response to precipitation treatments using partial distance-based redundancy analysis (partial db-RDA), a constrained ordination technique on the community dissimilarity matrix that removes the effect of a random variable or covariate before performing the RDA (Legendre and Gallagher 2001). This method is similar to partial linear regression, and is explain in detail in Legendre and Legendre (2012). We used the `capscale` function in the *vegan* package (Oksanen et al. 2013). Dissimilarity between sites was calculated using both binary (presence-absence) and quantitative (abundance) forms of the Bray-Curtis index. We first modeled the community response as a function of the year and treatment main effects and their interaction, accounting for the variation due to blocks. The effect of treatment and year main effects on community composition were determined using the standard pseudo-F test for the analysis (Legendre and Legendre 2012), with significance tested using a permutation test with 1000 permutations stratified within blocks. Denominator degrees of freedom are calculated by subtracting degrees of freedom from the random block variable (4 df), precipitation treatment (2 df), year (3 df), and the treatment by year interaction (6 df) from the total number of plots (48). We examined changes in diversity aboveground and belowground using species richness (S) and Shannon diversity (H') of stems in the four 0.25 m^2 quadrats per plot and buds in the ten 0.01 m^2 soil cores per plot, respectively. Shannon diversity was used because of its sensitivity to rare species.

2.3 Results

2.3.1 Productivity, Flowering Stem Density, and Stem Density

Drought reduced total (combined graminoid and forb) ANPP by 25 to 50% in drought plots relative to irrigated and ambient plots in 2009 and 2010 ($p < 0.01$, with no difference in ANPP among treatments in 2011 and 2012, the recovery years. The treatment by year interaction was driven by differences between drought plots and irrigated and ambient plots during the treatment years (2009 and 2010) but not during the recovery years (2011 and 2012; difference of differences = 18.9 g 0.25 m^{-2}, χ^2 = 18.1, df = 1, $p < 0.001$). Graminoid ANPP drove trends in total ANPP, with similar 25 to 50% reductions in ANPP in drought plots relative to ambient and irrigated plots during treatment years, but no difference among treatments during recovery years (difference of differences = 19.8 g 0.25 m^{-2}, χ^2 = 19.4, df = 1, $p < 0.001$; Fig. 2.3b). Forb ANPP was approximately 1/10 of graminoid ANPP and did not vary by precipitation treatment or year (Fig. 2.3c). Flowering stem density of C_4 grasses was reduced nearly 100% in drought plots relative to irrigated and ambient treatments in 2009 and was reduced 90% in drought plots relative to irrigated treatments in 2010 (Fig. 2.4). Like ANPP, the treatment by year interaction was driven by differences in flowering stem density between drought plots and irrigated and ambient plots during the treatment years (2009 and 2010) but similar flowering stem density during the recovery years (2011 and 2012; difference of differences = 13.2 stems 0.25 m^{-2}, χ^2 = 9.6, df = 1, p = 0.004).

Total, graminoid, and forb stem density varied by year, with no effect of precipitation treatments (Fig. 2.5b, d, f). The significant year effect might have been caused by the fire regime. In the year that plots were not burned (2010), total and graminoid stem densities were 15.92 ± 0.88 and 15.31 ± 0.88 stems per 0.01 m^2 for total and graminoid stems, respectively, but when plots were burned (2008, 2009, 2011, 2012) total and graminoid stem densities were 19.93 ± 0.49 and 18.97 ± 0.51 per 0.01 m^2, respectively (mean ± SE). Forb stem density was approximately 95%

less than graminoid density and varied by year but not treatment, averaging 0.96 ± 0.13 stems per 0.01 m^2 in years with fire and 0.61 ± 0.09 stems per 0.01 m^2 in 2010 (mean ± SE, Fig. 2.5f).

2.3.2 Bud Bank Density

Like stem densities, belowground bud bank densities were relatively insensitive to precipitation treatment. Graminoid bud bank density averaged 100-170 per 0.01 m2, but varied by both treatment and year (Fig 2.5a, c). Bud bank density in drought plots did not differ from ambient plots in either the treatment (2009-2010) or recovery years (2011-2012; Fig 2.5a, c)). Although densities tended to be greater in 2010 and 2011, graminoid bud density was significantly greater than drought and ambient plots only in 2012 (P = 0.016). Forb bud density averaged 7-25 per 0.01 m2 and did not vary significantly by year or treatment (Fig 2.5e).

2.3.3 Effects on Bud Bank Demographic Transitions

The number of buds per stem did not differ among treatments, but varied among years for total, graminoid, and forb groups (Fig. 2.7a, c, e). Pooled across all treatments and years, total buds per stem was 7.70 ± 0.17 (mean ± SE). Graminoid buds per stem was slightly lower, 7.27 ± 0.17, while forb buds per stem was higher but more variable, 17.54 ± 1.79 (mean ± SE).

The rate of bud transition to aboveground stem (stems per bud) did not differ among treatments, but varied among years for the total plant community and for graminoids (Fig. 2.7b, d). The lack of fire in 2010 caused lower rates of transition from bud to stem for graminoids, with 15.95 pm 0.57% of buds transitioning to stems in years with fire, but only 9.58 ± 0.58% transitioning to stems when plots were not burned in 2010 (mean ± SE). Neither precipitation treatment nor year affected forb transition rate from bud to stem (Fig. 2.7f), but transitions rates were more variable compared to graminoids, with 11.04 ± 1.57% (mean ± SE) of buds transitioning to stems. One outlier data point (2011, irrigated plot, stems per bud = 4.0) was excluded from the analysis because low numbers of buds caused a large value (Fig. 2.7).

2.3.4 Resistance and Resilience

We calculated resistance and resilience for aboveground responses, including ANPP, stem density, and C_4 grass flowering stem density, and for belowground bud bank density. There was no change in ANPP in response to irrigation, but in response to drought absolute ANPP declined over 25 g per 0.25 m^2 in 2009 and nearly 15 g per 0.25 m^2 in 2010, approximately 60 and 30% reductions, respectively (Fig. 2.8a). C_4 grass flowering stem density was nearly reduced to zero by drought, but flowering stem density changed little in response to irrigation (Fig. 2.8b). Both productivity and flowering were highly resilient following the drought, with no difference between drought and ambient plots the year immediately following the drought treatment (2011 and 2012). Stem density was highly resistant to drought, with no difference relative to ambient plots (Fig. 2.8c). Stem density tended to increase 20 to 25% in response to irrigation and remained elevated relative to ambient plots in the years following irrigation. Belowground bud density did not change in response to drought (Fig. 2.8d). Bud density did increase approximately 20% in response to irrigation, but not until 2010, the second year of irrigation. The increase in bud density persisted in 2011 and 2012 after the irrigation treatment ceased.

2.3.5 Plant Community Response

Redundancy analysis (db-RDA) on quantitative dissimilarity indicated differences in plant community composition among treatments both aboveground (canopy cover) and belowground in the bud bank (Fig. 2.9a, c). Aboveground, we observed differences among drought treatments ($F_{2,33}$ = 2.6, p = 0.004), but no year or treatment by year effects ($F_{3,33}$ = 1.2, p = 0.247; $F_{6,33}$ = 0.4, p = 1; respectively). Visual inspection of the ordination suggests that drought plots diverge from irrigated and ambient plots over time (Fig. 2.9a). Belowground, we observed effects of both treatment and year, but no interaction ($F_{2,33}$ = 1.9, p = 0.029; $F_{3,33}$ = 1.5, p = 0.051; $F_{6,33}$ = 0.7, p = 0.96; respectively). The ordination suggests that all three treatments started out relatively similar, but diverged slightly over time (Fig. 2.9c). We compared this analysis based on abundance dissimilarity to the analysis based on presence/absence of species to determine if divergence in plant communities in response to precipitation treatments was due to changes in the species present versus their abundance. Aboveground, community composition varied by both treatment and year, with no effect of their interaction ($F_{2,33}$ = 3.1, p = 0.001; $F_{3,33}$ = 3.0, p = 0.001; $F_{6,33}$ = 0.6, p = 1; respectively). We observed large differences between ambient plots and both drought and irrigated plots in all years (Fig. 2.9b), suggesting that aboveground changes were due to changes in plant species richness and abundance. Shifts in bud bank composition were due to change in abundance, as we found no effect of treatment, year, or their interaction on bud bank composition when dissimilarity was calculated using presence/absence ($F_{2,33}$ = 1.2, p = 0.247; $F_{3,33}$ = 0.9, p = 0.644; $F_{6,33}$ = 0.8, p = 0.774; respectively; Fig. 2.9d). Visual inspection of the db-RDA on belowground abundance estimated with live rhizome biomass in 2012 revealed apparent separation of treatments, but the quantitative analysis indicated that differences among treatments were not significant (Fig. 2.10). Further analysis revealed few changes in abundance of individual species in response to treatments, although both bud bank density and canopy cover of *Sorghastrum nutans* tended to decline slightly in response to drought relative to ambient and irrigated treatments (data not shown).

Above- and belowground species richness did not vary by treatment or year (Fig. 2.11a, b). Treatment and year interactively affected belowground Shannon diversity (H'). Post-hoc analysis of the interaction confirmed that while irrigated and drought plots had greater diversity that ambient plots in 2011, diversity of irrigated and drought plots was less than the diversity of ambient plots in 2010 ($p < 0.001$, Fig. 2.11c). Aboveground, Shannon diversity also had a significant treatment by year interaction (Fig. 2.11d). Drought plots were more similar to ambient plots in 2009 and 2010, but more similar to irrigated plots in 2011 and 2012. An interaction contrast of drought plots versus ambient and irrigated plots in 2009 and 2010 versus 2011 and 2012 confirmed this change in diversity of drought plots relative to other treatments (emphp = 0.003,Shannon diversity in 2010 was less than in all other years.

2.4 Discussion

We show that bud bank density, demography, and composition are insensitive to drought, allowing rapid recovery of the aboveground plant community following drought. These findings have important implications for our understanding of grassland responses to drought, in particular the potential importance of the timing of drought for resilience of plant community composition and productivity. We observed above- and belowground shifts in plant community composition in response to precipitation treatments that were driven primarily by changes in species abundance. Productivity and C_4 grass flowering suffered large declines in response to drought, but recovered to ambient levels immediately after favorable conditions resumed. Bud bank density and demography did not change in response to drought, but bud density tended to increase in response to irrigation.

2.4.1 Sensitivity of Bud Bank Demography

Precipitation treatments did not affect bud bank demography. Neither the transition from bud to stem nor bud natality (production of new buds) varied by treatment. The insensitivity of bud bank demography to drought was reflected in the stability of stem density and bud density. Both were highly resistant when perturbed with severe drought, with no difference between drought and ambient plots in any year during or following the treatment. Koerner et al. (2013) also found that stem density was highly resistant to change in precipitation regime in ungrazed plots.

We found that bud and stem densities were more sensitive to favorable conditions (irrigation) than to drought. When moisture stress was eliminated with irrigation, bud and stem densities tended to increase relative to ambient conditions, but we observed no change due to drought treatment. This suggests that the tallgrass prairie plant community is resistant to changes in numbers of individuals when perturbed by drought, but can increase numbers of stems under favorable conditions. The belowground perennating organs that bear buds, including various modifications of belowground stems (and some roots), represent a reserve for recruitment of new aboveground individuals after disturbance (Klimešová and Klimeš 2007). Plants can thereby replenish numbers of ramets aboveground by recruiting new stems from buds. The continued growth of new stems may be controlled by different factors than those that stimulated the transition from belowground bud to aboveground stem.

Several factors are known to influence the transition from bud to stem. In unburned tallgrass prairie the accumulated detritus layer insulates the soil from warming by solar irradiation relative to burned prairie, delaying tiller emergence from dormant buds (Knapp and Seastedt 1986). However, detritus not only delays tiller emergence but also lowers tiller density relative to burned prairie (Hulbert 1969). We observed the rate of transition from bud to tiller in the year our plots were unburned (2010) was half the transition rate during burned years. The light cues that signal dormant buds to emerge as tillers may be altered or intercepted by the detritus layer in unburned prairie. The work of Deregibus (1983, 1985) suggests that the red/far-red light ratio may signal tiller emergence in *Lolium* species, while other studies have investigated the possible role of nitrogen as another important cue (Bonser and Aarssen 2003, Dalgleish et al. 2008). Williamson et al. (2012) tested the model of Tomlinson and O'Connor (2004) and found that while C_3 grasses responded as predicted to light and nitrogen availability, C_4 grasses did not. When water is not limiting, the primary controls over bud transition to tillers in tallgrass prairie are most likely temperature (Knapp and Hulbert 1986, Ott 2009), light (Deregibus et al. 1983, Tomlinson and O'Connor 2004, Williamson et al. 2012), and nitrogen (Tomlinson and O'Connor 2004).

The timing of drought stress may explain the insensitivity of bud bank demography to drought. Buds of C_4 grasses, which account for over 80% of total buds in this study, transition to stems primarily in the spring (April and May), followed by rapid production of the majority of that season's buds as those tillers grow (Ott and Hartnett 2012). This period of production of tillers and buds largely concludes before periods of high water stress in mid-summer, which is the normal pattern for this region. Therefore, we hypothesize that in order for drought to affect the transition from bud to stem or the production of new buds, it must coincide with the primary period when buds are cued to transition to stems. This rapid production of buds early in the growing season may be a strategy to protect against meristem limitation the following growing season.

Although our drought treatment began in March, at the start of the emergence of stems, deep soil moisture most likely buffered plants from immediately experiencing drought stress. Long-term drought stress may be needed to affect bud bank demography. Evans et al. (2011) found that large declines in the abundance of the dominant grass in semi-arid shortgrass steppe of western North America did not occur until at least the 4th consecutive year of experimental growing season drought. Our results suggest that the shortgrass steppe plant community may have resisted change until belowground bud bank reserves were depleted.

During the two years of experimental manipulation of precipitation in 2009 and 2010, ambient precipitation was average or slightly above average. However, rainfall was below average during following two growing seasons of recovery from experimental manipulations (2011 and 2010). In response to this natural drought, treatments responded similarly, with reductions in productivity, C_4 grass flowering stem density, and stem density, but relatively little change in bud bank density. In

2012 there was some divergence among treatments in bud bank density, suggesting that the legacy of experimental severe drought may not be realized immediately, but may persist for multiple years after conditions improve.

2.4.2 Contribution of the Bud Bank to Resilience

This study and others demonstrate that moisture availability is a primary factor controlling plant productivity (Briggs and Knapp 1995) and C_4 grass flowering (Knapp and Hulbert 1986) in tallgrass prairie. ANPP and C_4 grass flowering stem density were not resistant to drought, with 30 to 60% reductions in ANPP and nearly complete absence of C_4 grass flowering during drought treatments. However, both ANPP and C_4 grass flowering stem density were highly resilient when favorable conditions returned, quickly rebounding to match or exceed ANPP and flowering of ambient controls. Previous research has shown that bud bank density increases with mean annual precipitation across a broad gradient of mean annual precipitation in the Great Plains (Dalgleish and Hartnett 2006). Greater bud bank density also corresponds to greater interannual variability in ANPP (Knapp and Smith 2001), suggesting that large populations of belowground buds prevent meristem limitation and allow grasslands to opportunistically capitalize on periods of high water availability. This sensitivity of ANPP to precipitation may be driven by the stability of the bud bank, as tallgrass prairie consistently maintains a large reserve of meristems under severe drought.

Many previous studies only consider aboveground responses such as productivity when studying the effects of diversity on resistance, resilience, and recovery (Carter and Blair 2012, Halpern 1988, Klimeš et al. 2013, van Ruijven and Berendse 2010, Vogel et al. 2012). Though such studies contribute to our understanding of the factors influencing ecosystem function, they may provide limited insight into the mechanisms that control resistance and resilience of populations of plants. If moisture stress does not occur at the key time, cues for bud release into stems may be disconnected from the factors that control continued aboveground plant growth.

2.4.3 Plant Community Change in Response to Drought

The shifts in community composition we observed were primarily driven by changes in species abundance rather than species turnover over the time frame examined. Observations from the widespread drought on the Great Plains of central North America in the 1930s indicated that species turnover becomes much more important as the length and severity of drought increase (Albertson and Weaver 1942, Weaver and Albertson 1936). The changes we observed in community structure may be the result of individual species response to water stress (Tucker et al. 2011), altered intra- and interspecific interactions (Chesson and Huntly 1997), or stochastic variation in species demography. Craine et al. (2011) suggest that because plant species inhabiting relatively mesic lowland tallgrass prairie suffer the greatest reductions in canopy cover during drought, they will be preferentially lost with projected warming and drying. However, our results indicate that species typically associated with mesic habitats may be more resilient than previously thought, as reductions in plant size (cover and biomass) do not necessarily lead to changes in the number of individuals (stem and bud density) or composition of plant species in the community in response to short-term drought.

2.5 Conclusions

Our results demonstrate that bud bank populations in tallgrass prairie are highly resistant to drought over the time period examined. We found that bud bank demography, including the transition from bud to stem and the number of buds per stem, was more sensitive to increased water availability than to drought. These results suggest that bud banks play a general role in buffering grassland plant population and community dynamics during disturbance, serving as a reservoir for recruitment of future aboveground stems when favorable conditions return.

Future work should investigate how the timing of drought affects tallgrass prairie plant community dynamics. In particular, moderate to severe moisture stress during key spring and early summer periods of bud outgrowth and bud formation by new stems may disproportionately affect the plant community relative to drought at other times during the growing season. Moreover, in tallgrass prairie, where mid-season drought has been historically common, projected increases in the length of time between large rain events (Knapp et al. 2008) during mid-summer may force less change in the plant community relative to drought early in the growing season. Bud bank density may control the rate and magnitude of plant community recovery following disturbance. Experimental manipulation of bud bank density, followed by disturbance and recovery, could test the role of bud bank density when mean annual precipitation is constant.

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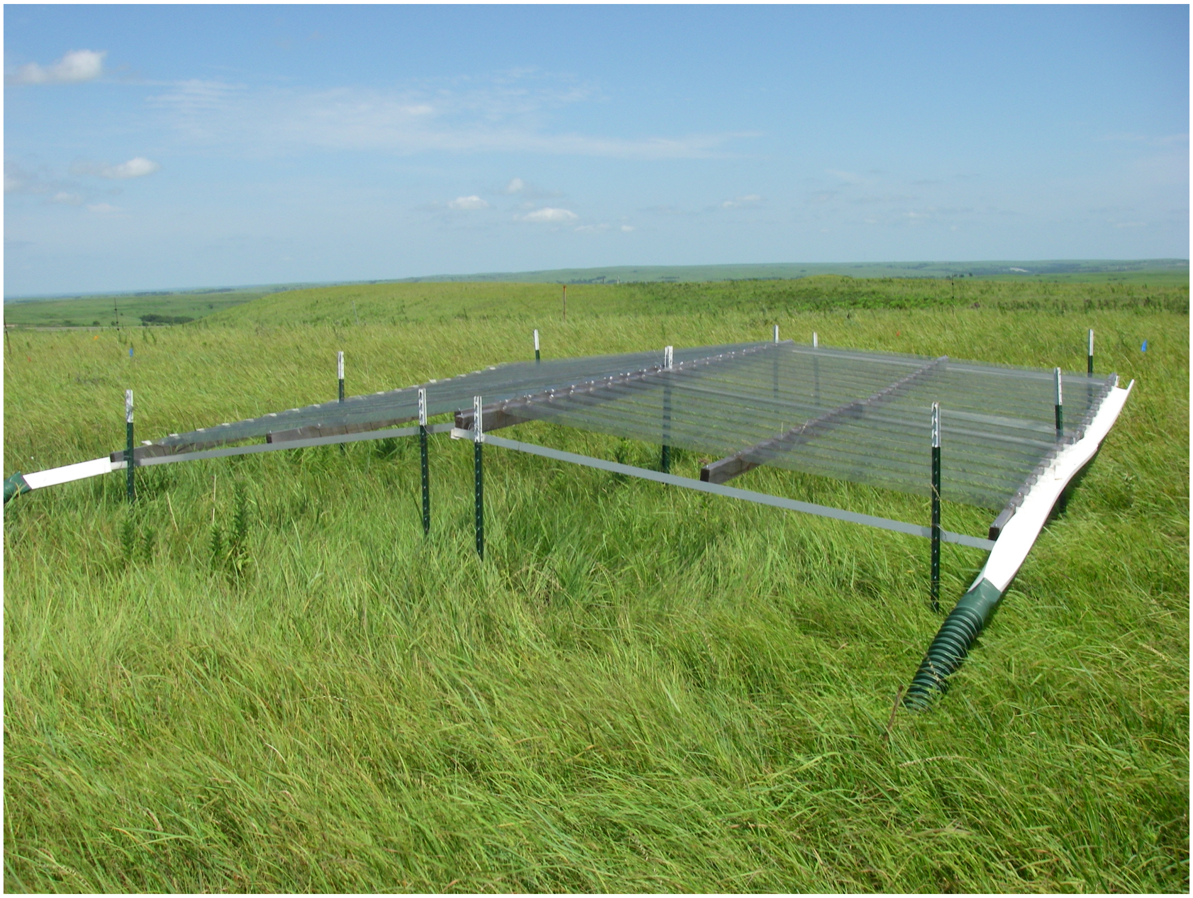

Figure 2.1: A 4.5 × 4.5m rainfall interception shelter, with shingles spaced to cover 80% of the total shelter area. Picture taken 21 July 2010 by B. VanderWeide.

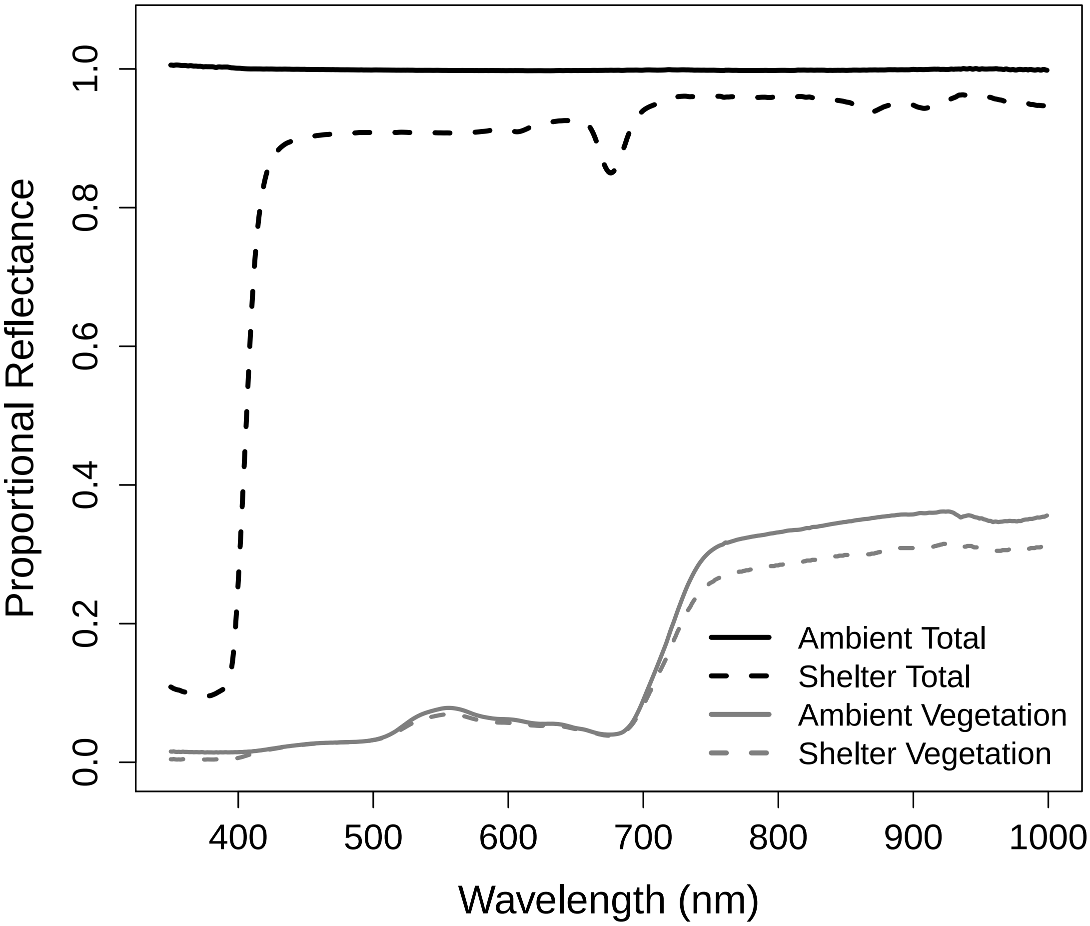

Figure 2.2: Reflectance of solar radiation underneath and outside rainfall manipulation shelters, with reflectance of vegetation underneath and outside for comparison. “Shelter” indicates reflectance after light has passed through the Lexan®shingle material, while “ambient” indicates reflectance with no interception.

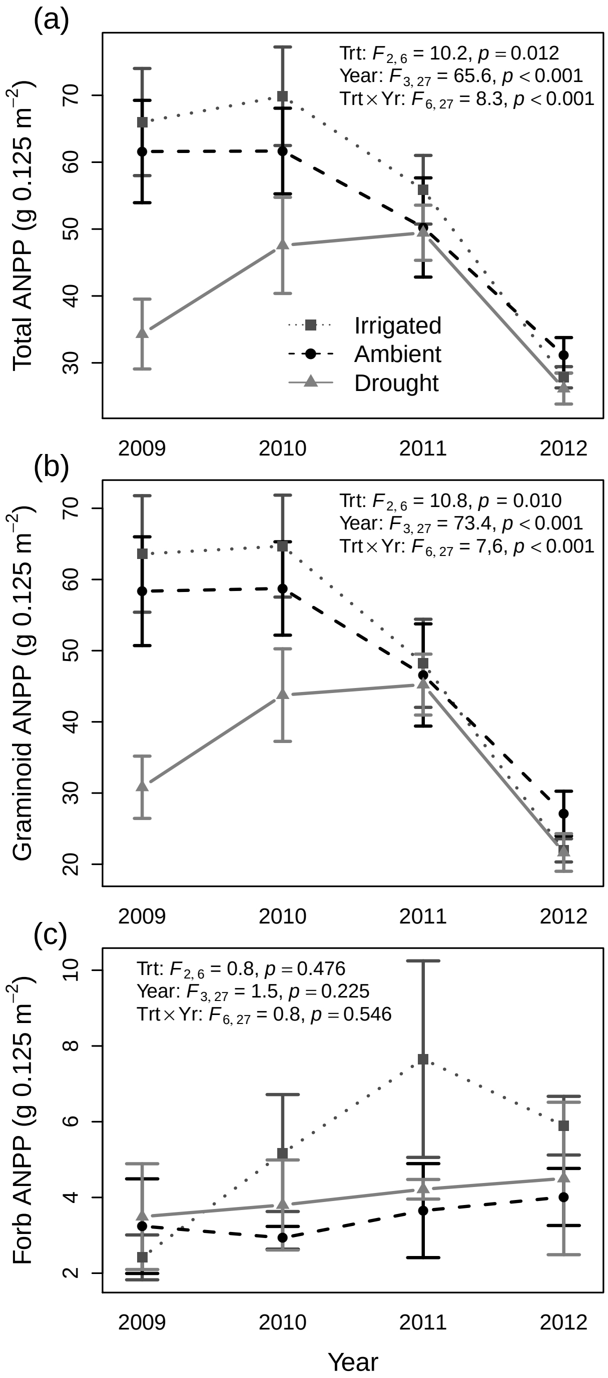

Figure 2.3: Aboveground net primary productivity (ANPP) of treatments from 2009 to 2012 for (a) all species combined, (b) graminoid species, and (c) forb species. Precipitation treatments were imposed in 2009 and 2010, while plots recovered in 2011 and 2012. Points are means ± 1 SE.

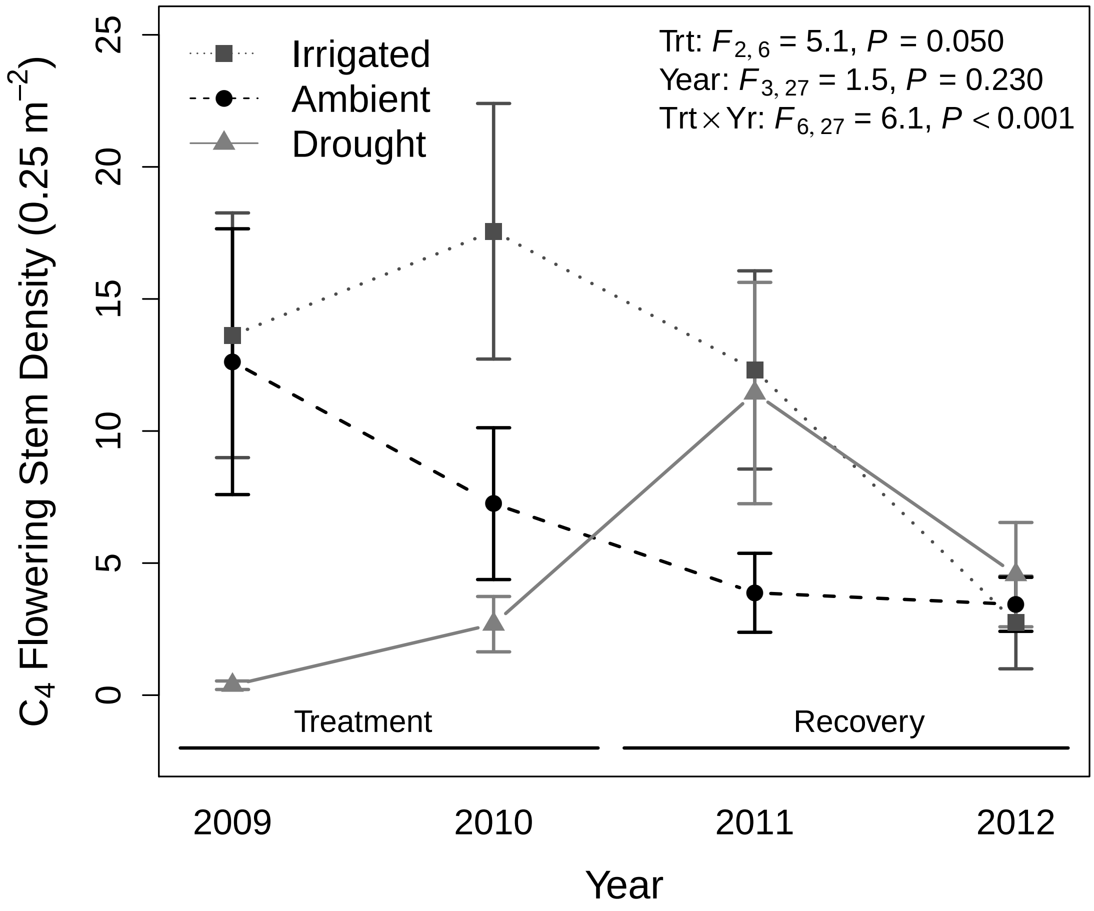

Figure 2.4: Density of C_4 grass flowering stems during the treatment (2009-2010) and recovery (2011-2012) periods. Points are means ± 1 SE.

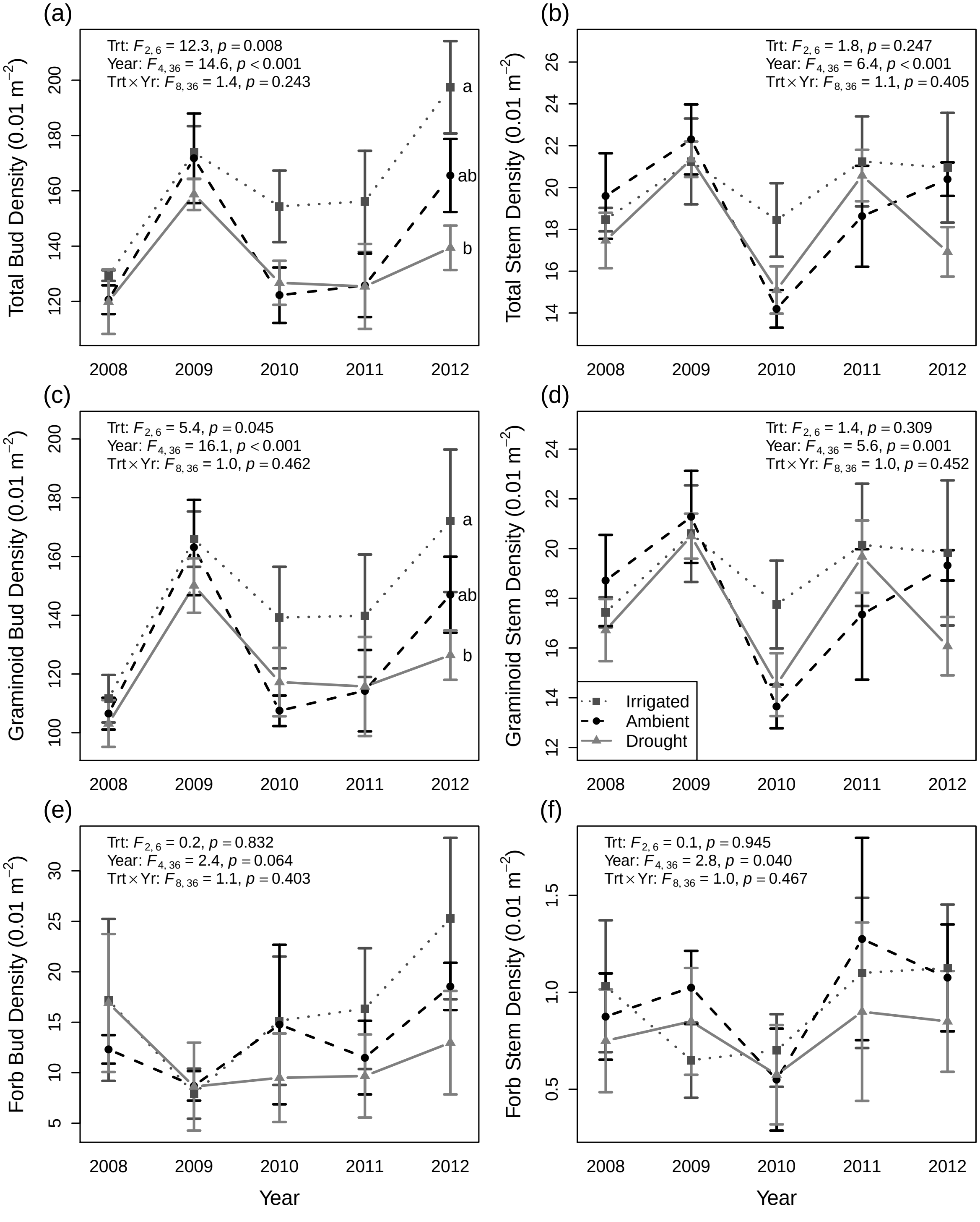

Figure 2.5: Bud and stem density responses before precipitation treatments (2008), during treatments (2009-2010), and following treatments (2011-2012). Bud densities are in the left column and stem densities are in the right column. Total bud (a) and stem (b) densities are in the first row, graminoid densities in the second row (c, d), and forb densities in the third row (e, f). Points are means ± 1 SE. Lowercase letters indicate significant differences between treatments within years ($p < 0.05$).

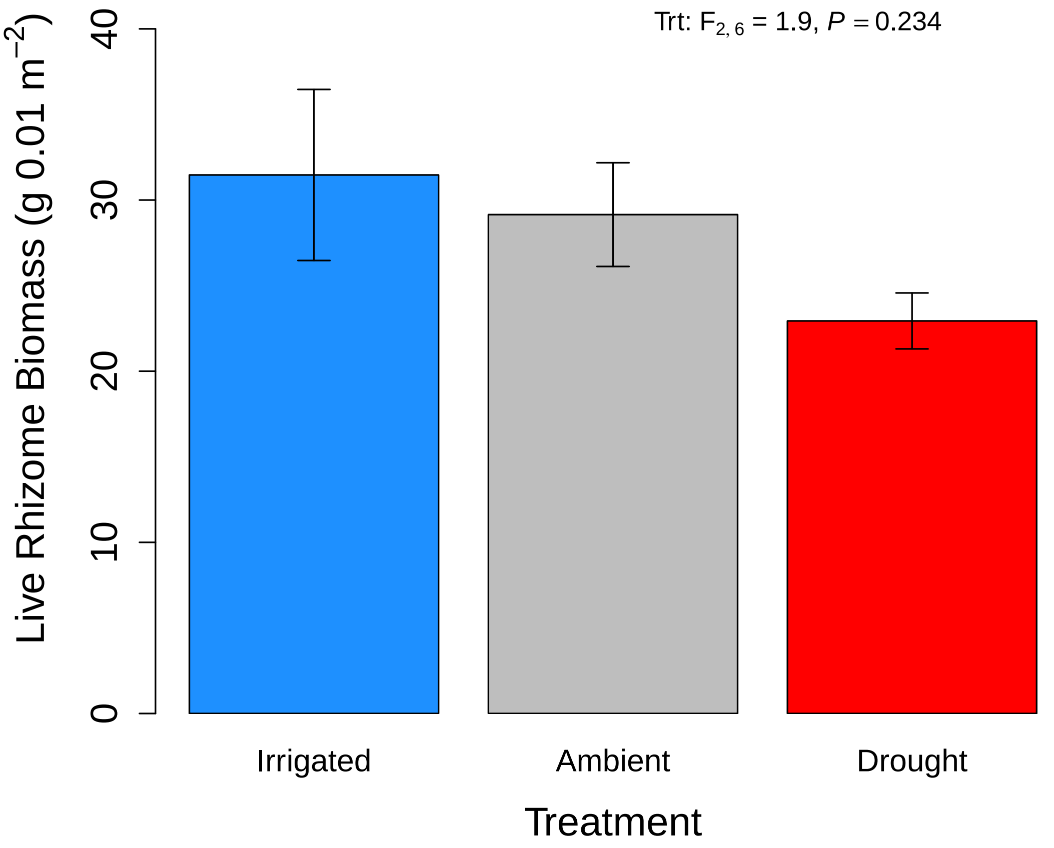

Figure 2.6: Biomass of live rhizomes in 0.01 m^2 soil cores, sampled after two years of recovery (in 2012). Points are means ± 1 SE.

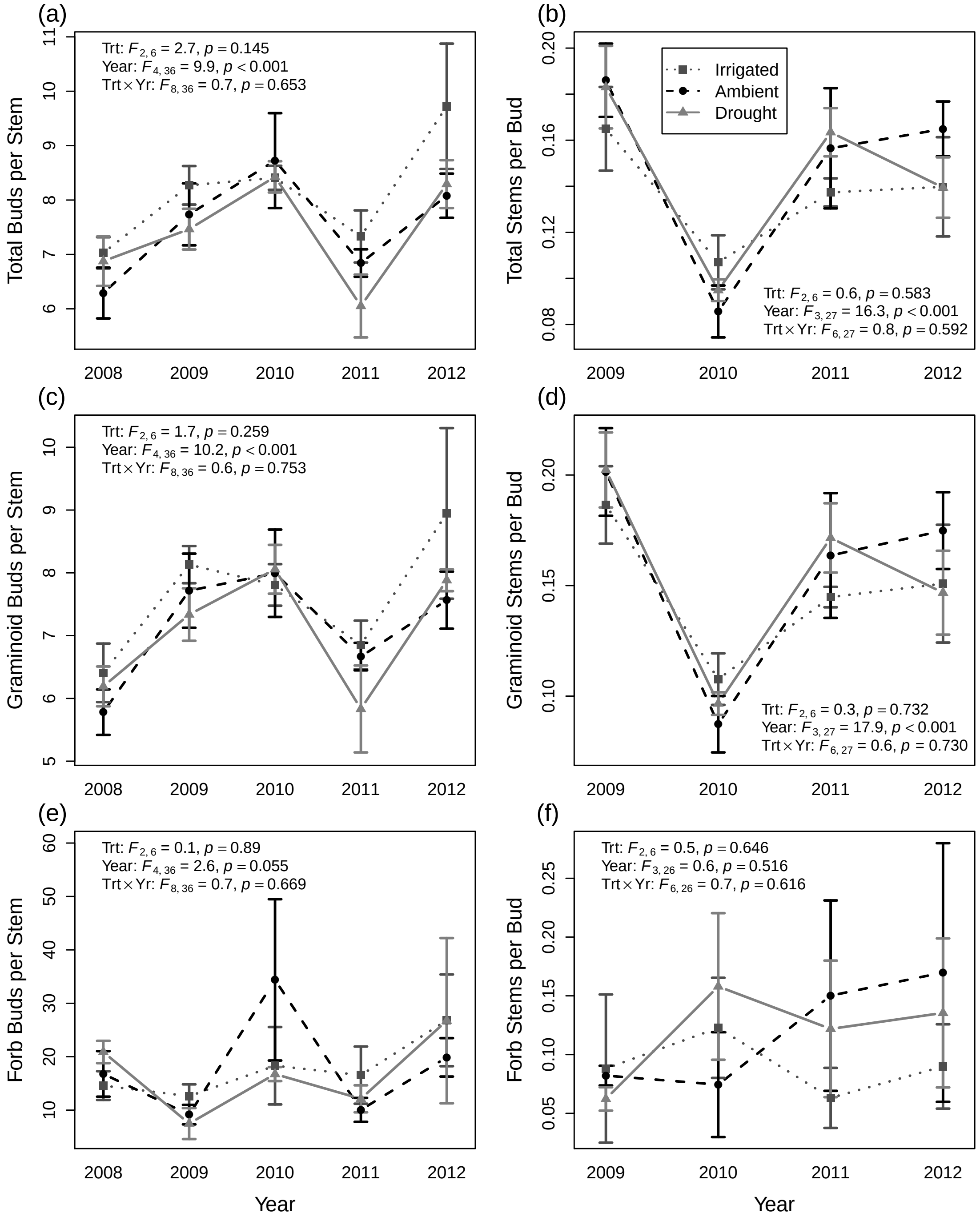

Figure 2.7: Bud bank demographic transitions before precipitation treatments (2008), during precipitation treatments (2009-2010), and following precipitation treatments (recovery, 2011-2012). Buds per stem are in the left column and stems per bud in the right column. Stems per bud could not be calculated for 2008 because bud bank density was not sampled in 2007. Total transitions are in the first row (a, b), graminoid transitions in the second row (c, d), and forb transitions in the third row (e, f). In (f), an outlier was removed from the plot to improve clarity (2011, irrigated plot, forb stems per bud = 4.0). However, including the outlier had no effect on interpretation. Points are means ± 1 SE.

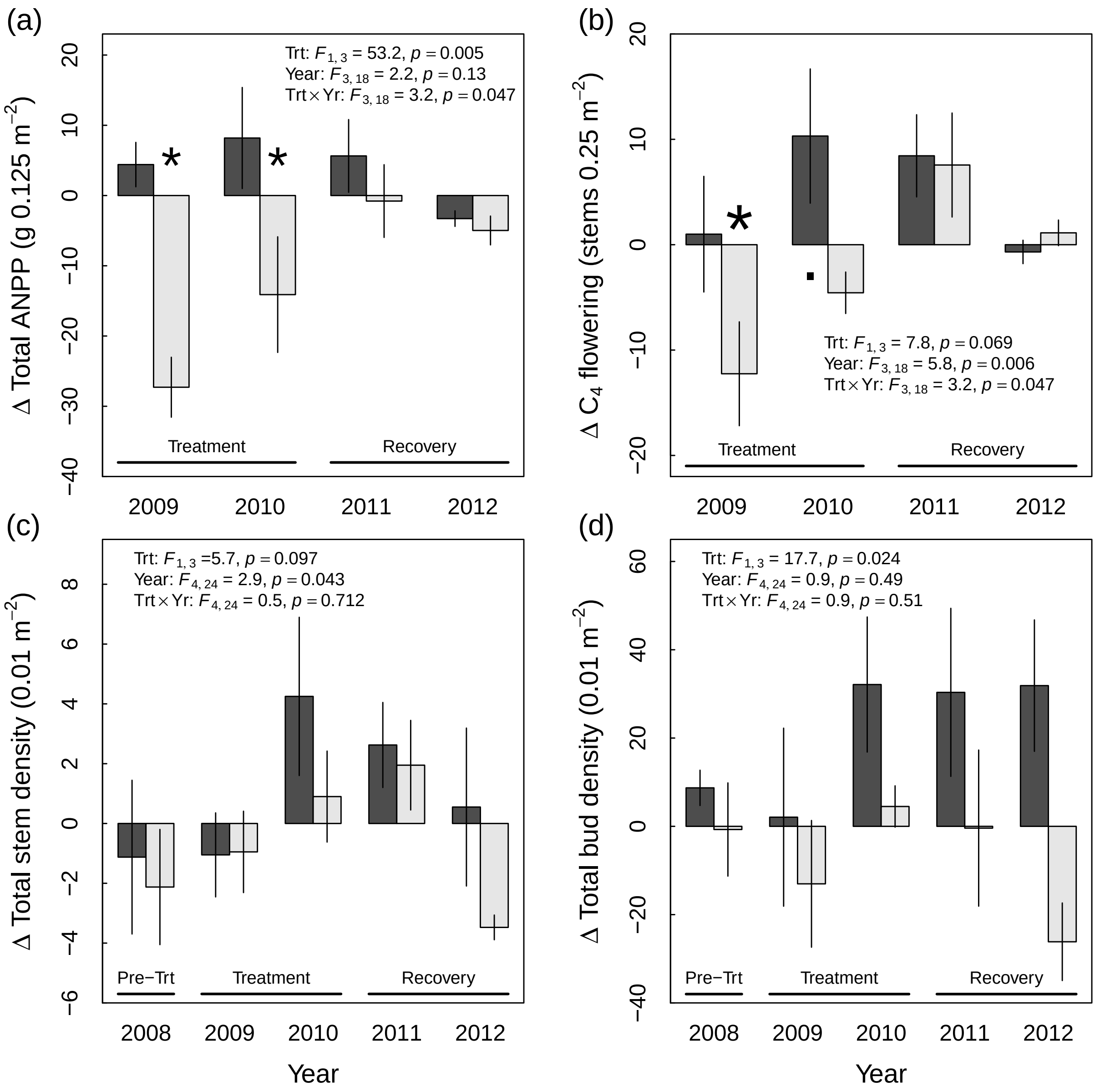

Figure 2.8: Resistance (2009-2010) and resilience (2011-2012) of absolute responses in all years, calculated as the difference of treatment responses from the ambient control. Dark gray bars indicate irrigated treatment, and light gray bars indicate drought treatment. (a) Total aboveground net primary productivity (ANPP), (b) C_4 grass flowering stem density, (c) total stem density, and (d) total belowground bud bank density. Bud densities in 2008 are pre-treatment. Asterisks indicate significant differences from zero at $p<0.05$, while $(\cdot)$ indicates a significant difference from zero at $p<0.1$. Bars indicate the means, with error bars ± 1 SE.

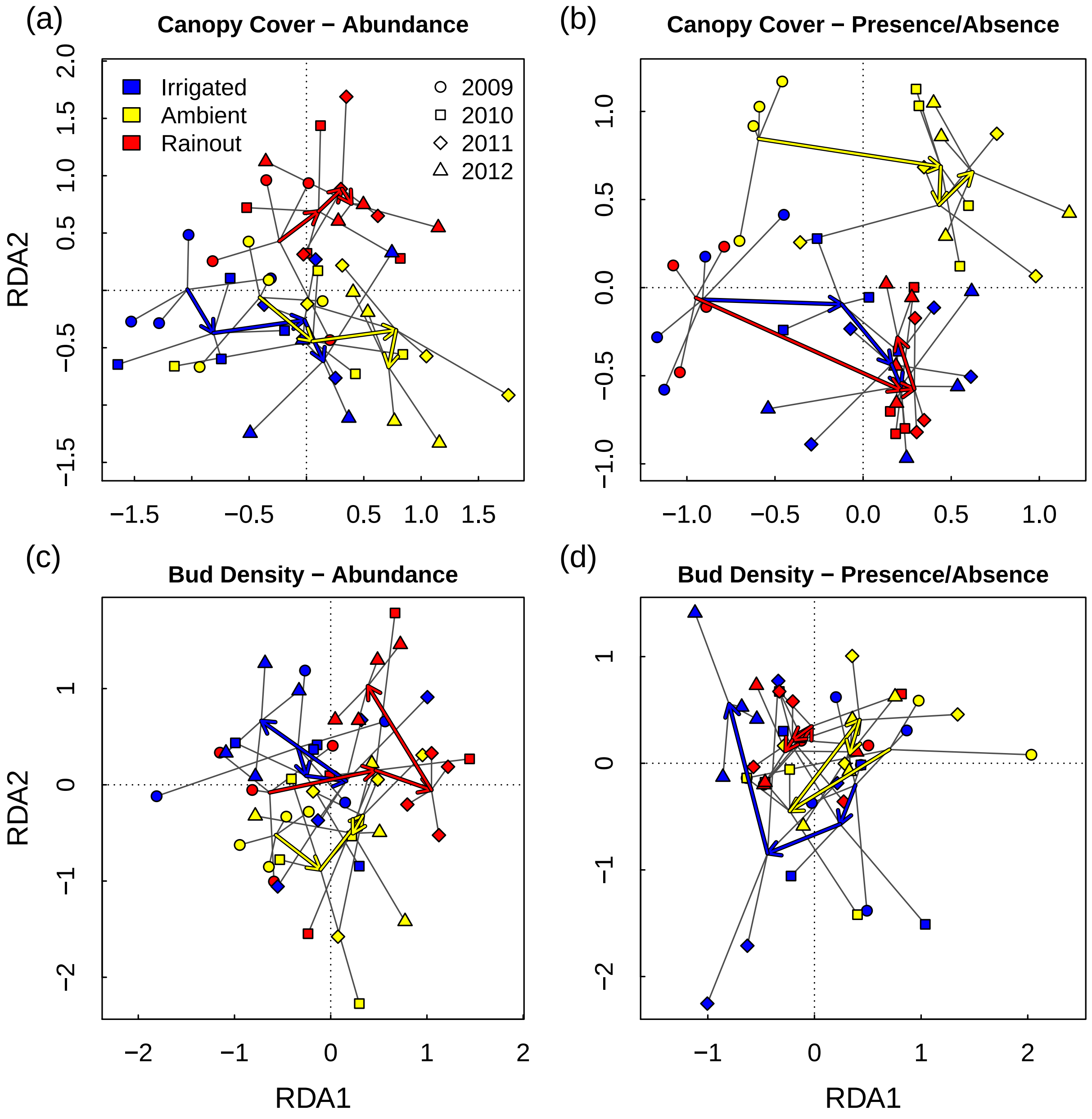

Figure 2.9: Partial distance-based redundancy analysis (db-RDA) ordination showing the first two axes of (a,b) canopy cover and (c,d) bud bank density from 2009 to 2012. For the left column, distances were calculated using quantitative (abundance) Bray-Curtis dissimilarity, while for the right column distances were calculated using binary (presence-absence) Bray-Curtis dissimilarity. Symbols represent sites within years, and gray lines connect plots treated similarly within a year to their centroid. Arrows connect centroids of treatments across years, with the base of the arrow at year *t* and the tip of the arrow at year (*t*+1).

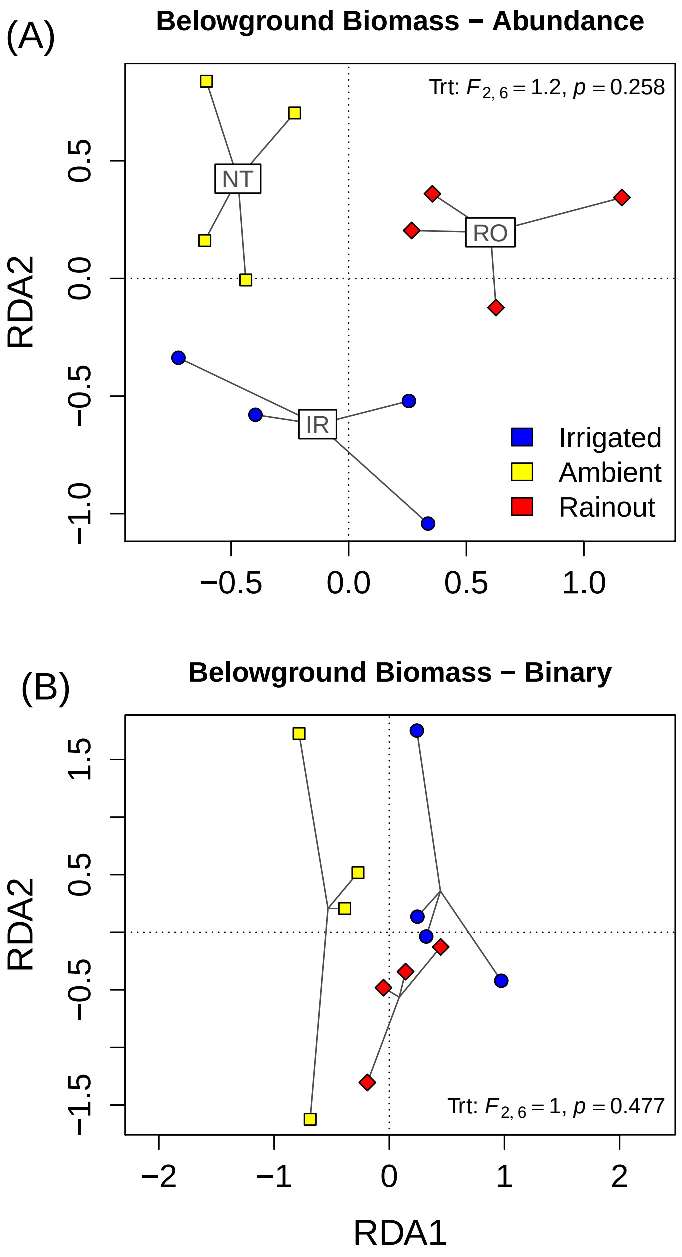

Figure 2.10: Partial distance-based redundancy analysis (db-rRDA) ordination of the belowground plant community, with abundance estimated using biomass of live rhizomes in 2012. Part (A) was calculated using quantitative (abundance) Bray-Curtis dissimilarity, while (B) was calculated using binary (presence-absence) Bray-Curtis dissimilarity. Symbols represent sites, and gray lines connect sites with the same treatment to their centroid.

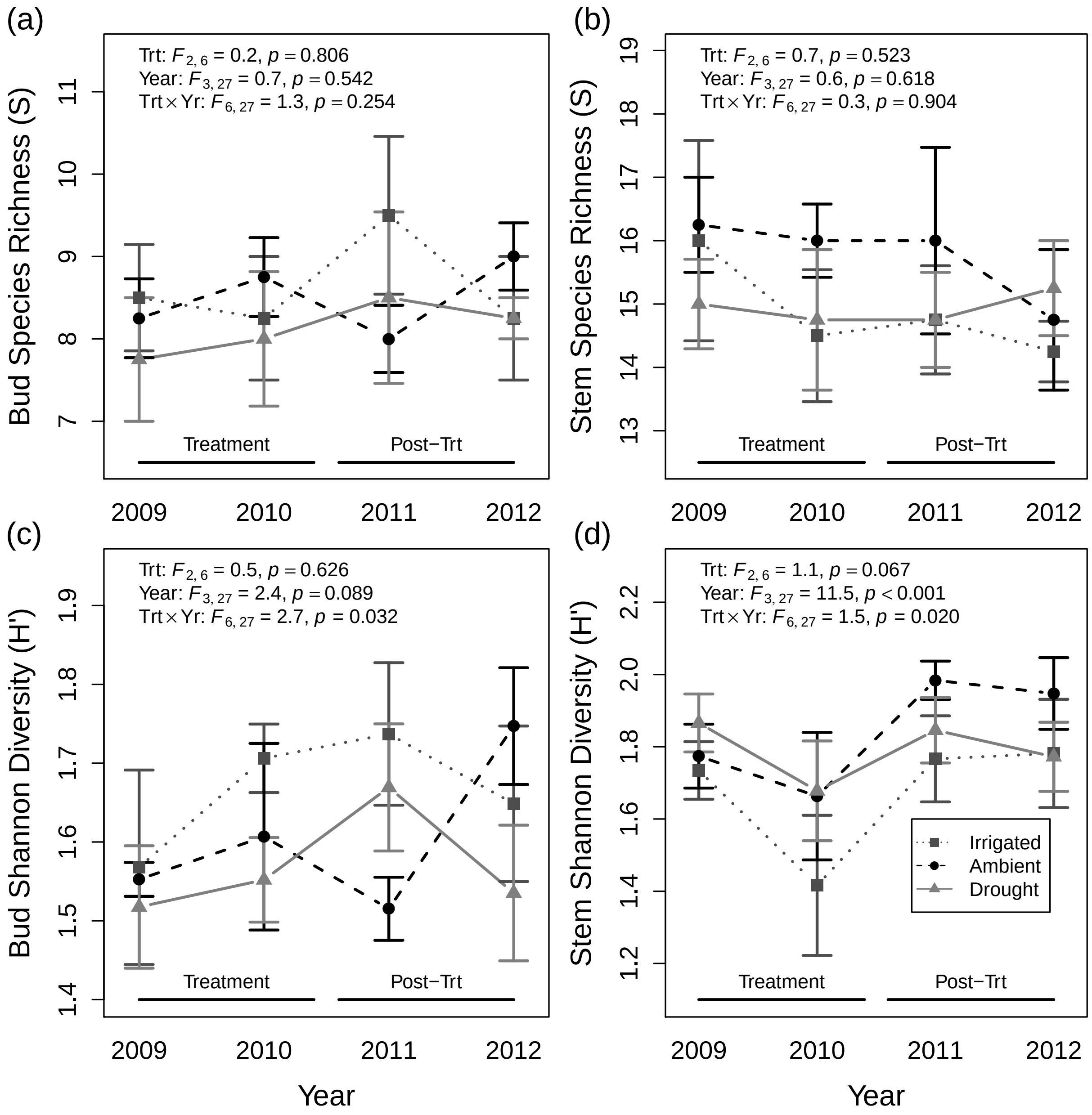

Figure 2.11: Change in species richness (S, top row) and Shannon diversity (H', bottom row) for the bud bank (a, c) and stems (b, d). Points are means ± 1 SE.

Chapter 3

Legacy effects of grazing in tallgrass prairie: the role of bud and seed banks in vegetation dynamics

Abstract

The dynamics of tallgrass prairie plant communities have historically been driven by the interacting effects of grazing by large ungulate herbivores, frequent fire, and a variable mid-continental climate. The effects of grazing on tallgrass prairie are well known and include decreased C_4 grass abundance, increased forb abundance, increased species diversity, and increased spatial heterogeneity. However, the legacy effects that persist after grazer exclusion are not well known, despite implications for land management, biodiversity conservation, and understanding basic ecological processes. In this study I examined the above- and belowground responses of the plant community and soil nutrients following release from persistent grazing. I monitored aboveground net primary productivity, canopy cover, aboveground stem density, belowground bud bank density, and seed bank density in ungrazed, grazed, and recovering (last grazed in 2007) areas of upland tallgrass prairie. I also sampled soil potassium, phosphorus, inorganic nitrogen, total nitrogen, and total carbon in spring 2013, six years after grazers were removed from recovery areas. Within four years of grazer exclusion, the aboveground plant community became more similar to ungrazed prairie for all responses, including stem density, diversity, and community composition. Belowground, however, the density, diversity, and composition of the bud bank after four years of grazer exclusion remained more similar to the bud bank in grazed than ungrazed areas. Immediately after grazers

were removed, the number of buds per stem and stem per bud were most similar to grazed areas, but over the four years of this study become more similar to ungrazed areas. Thus bud bank demography may be an important link between the above- and belowground plant communities. The response of the soil seed bank resembled the response of the bud bank, with density and diversity more similar to grazed areas than ungrazed areas. However, species composition of the seed bank in recovery areas differed from both grazed and ungrazed areas. Responses of soil nutrients to removal of grazers varied, and in some cases was affected by fire history the previous year. These results suggest that after release from grazing, the response of tallgrass prairie plant communities differs above- and belowground. Differences in the rate of recovery may be explained by the relative turnover rate of individuals in plant populations and rates of nutrient cycling.

3.1 Introduction

Disturbances leave a persistent impact on ecological processes long after the perturbation has ceased (Foster et al. 2003). These land-use legacies can persist for years to centuries, affecting ecosystem function and the ability of the system to respond to future disturbance (MacDougall et al. 2013). By understanding what the disturbance legacies are and how they persist, we can adopt management strategies that will enhance resilience of ecosystems to protect against current and future changes in land management and climate.

Grazing by large ungulate herbivores affects both the abiotic and biotic components of tallgrass prairie. Feeding and non-feeding behaviors of grazers increase the spatial heterogeneity of tallgrass prairie vegetation and other grasslands (Adler et al. 2001), often accompanied by changes in species diversity (Bakker et al. 2003). Physical disturbance and compaction of the soil by grazers changes the soil structure and hydrology (Knapp et al. 1999). Grazers redistribute nutrients through the system through their dung and urine, leading to patches of high nutrient availability (Knapp et al. 1999). In some cases, grazing causes woody encroachment or desertification in grasslands (Asner et al. 2004). Preferential grazing of certain functional groups differentially alters plant species access to nutrients, water, and light, causing shifts in competition for limiting resources (Augustine and McNaughton 1998, Hartnett et al. 1996). As a result of these shifts in competitive hierarchies, plants shift their relative energy allocation to sexual and vegetative reproduction.

Many plant species produce propagules that persist in the soil for several years (Baskin and Baskin 1998, Ott and Hartnett 2012), leaving "memory" of the disturbance in plant populations.

Despite the important of belowground plant responses to disturbance, many studies focus on aboveground responses when assessing responses to perturbation. In perennial herbaceous plant communities such as grasslands, aboveground vegetation dies back to the soil surface each year during periods of seasonal drought or cold temperatures, and regeneration of the aboveground plant community the following growing season depends entirely emergence from stored propagules in the soil (Benson and Hartnett 2006). Since regeneration of aboveground stems depends entirely on emergence from belowground propagules, belowground responses of plants may better predict the overall plant community response to disturbance than responses of the aboveground vegetation, which is replaced annually. Understanding patterns and mechanisms of plant community responses to disturbance, and the legacy effects during recovery, requires documenting changes in plant community abundance in these difficult-to-observe parts of the plant community (Marks and Mohler 1985, Rogers and Hartnett 2001, Van Der Valk and Verhoeven 1988). Moreover, this approach may help explain why abundance of some plant species changes quickly with disturbance, while abundance of other species remains stable.

Population-level processes control many grassland responses to grazing, including the relative contributions of vegetative and sexual reproduction to plant community response and recovery. Seed banks contribute little to the aboveground plant community in ungrazed, annually burned tallgrass prairie, where more than 99% of established aboveground stems originate from the belowground population of meristems associated with rhizomes and other perennating organs, which Harper (1977) referred to as the "bud bank" (Benson and Hartnett 2006). In relatively small-scale disturbance from pocket gophers (*Geomys bursarius*), the bud bank can also account for the majority of recolonizing stems (Rogers and Hartnett 2001), although the seed rain and seed bank also contribute significantly to recruitment in sites disturbed by pocket gophers. Grazing by large ungulate herbivores enhances the diversity of grassland plant communities, facilitating increased relative abundance of annual and short-lived perennial plant species that establish primarily from the seed bank (Hartnett et al. 1996). However, even in grazed areas most aboveground stems continue to be recruited from belowground buds on rhizomes and other plant organs (Dalgleish and Hartnett 2009).

Soil seed banks enable species to disperse temporally, allowing populations to persist during periods that are not favorable for growth and reproduction (Harper 1977, Salguero-Gomez et al. 2012). The density and composition of the soil seed bank is determined in part by the abundance of species with seeds that can be "banked," or stored, in the soil for at least one year. The dominant perennial C_4 grasses in tallgrass prairie do not have a long-term soil seed bank, while many of the sub-dominant annual and short-lived perennial forbs and grasses do form soil seed banks (Rabinowitz 1981). Because grazing suppresses the dominant C_4 grasses in tallgrass prairie and promotes greater abundance of sub-dominant grasses and forbs, grazing should increase seed bank density.

Seed banks should be considered when determining land management strategies. Soil seed banks may allow native plant species to persist following invasion of invasive exotic plant species (Robertson and Hickman 2012). Restoration practitioners often rely on soil seed banks to contribute to regeneration of desirable states following management actions, or may need to pursue alternative restoration practices if the seed bank is dominated by non-desirable species (Bakker et al. 1996, Suding et al. 2004). Despite the importance of the seed bank in plant community dynamics and implications for land management, I am not aware of any work that explores both the response of grassland seed banks to grazing and their contribution to recovery following grazing.

Until recently few studies had examined the role of bud banks in population dynamics (but see Dalgleish et al. 2008, Klimešová et al. 2011, Ott and Hartnett 2012). Fewer studies have examined the role of bud banks in plant community response to and recovery from grazing, though some have explored bud bank dynamics of individual species (Busso et al. 1989, N'Guessan and Hartnett 2011) and the role of clonal growth in grazing resistance (Brathen and Junttila 2006). Some effects of persistent grazing may be mediated through bud bank demography. Plant responses to herbivory, including tolerance, defense, and escape, may rely on changes in the production of new buds, growth of buds into aboveground stems, or changes in the timing of these events. Since the dominant prairie grasses evolved under grazing pressure, these grasses protect their buds belowground to limit grazing damage and facilitate rapid regrowth (Lehtila 2000, Nilsson et al. 1996), and permit changes in plant architecture by releasing buds that grow into low, prostrate stems that are inaccessible to grazers (N'Guessan and Hartnett 2011).

Grazing may affect bud bank demographic transitions by altering conditions within and around a plant. Bud outgrowth into tillers is affected by soil temperature (Knapp and Hulbert 1986), light quality/quantity (Deregibus et al. 1985), soil nutrients (Tomlinson and O'Connor 2004), and release from apical dominance, all factors that are altered by feeding and non-feeding grazer behavior. Grazing may reduce grass bud bank density by stimulating high rates of transition from bud to tiller (Dalgleish and Hartnett 2009), or by limiting the growth of new buds (bud natality) by reducing overall plant growth. Similarly, bud density may increase when grazers are removed because fewer buds are growing out into tillers, or because more buds are being produced per stem.

Few earlier studies have examined recovery from grazing, and little is known about the responses of belowground parts of the plant community, the bud and seed banks, when persistent grazing is removed. In this study my primary goal was to document the persistent impacts of grazing on vegetation and nutrients, and to assess the contribution of the bud and seed banks to plant community response to and recovery from grazing. The following questions guided my research:

1. When grazers are removed, do bud bank density and bud bank demographic rates quickly return to states characteristic of ungrazed areas? Grazing may affect production of new buds by stems, or outgrowth of buds into aboveground stems by altering light, soil temperature, water availability, and nutrients. Without grazers affecting these resources, does the bud bank quickly become more like that of ungrazed areas? *H1*: When grazers are removed, bud bank density and demographic rates in recovery areas will quickly resemble the density and rates of ungrazed areas.

2. How does seed bank density and composition change after grazers are removed? Grazed areas have the greatest relative abundance of annuals and short-lived species that form persistent soil seed banks, and ungrazed areas have the lowest relative abundance, so density during recovery many "decay" slowly to the ungrazed state. *H2*: Seed bank density will be

greatest in grazed areas, least in ungrazed areas, and intermediate in recovery areas as seeds are lost from the seed bank.

3. How do plant community composition and species diversity respond during recovery from persistent grazing? Are changes in diversity driven primarily by changes in the number of species present (richness), or changes in their relative abundance (evenness)? *H3*: Changes in diversity during recovery will be driven by changes in relative abundance of species in both the bud and seed banks, with most species persisting in the plant community due to their long-lived propagules.

4. Do aboveground (biomass, stem density) and belowground (bud bank, seed bank) plant responses differ in their recovery? *H4*: Since buds and seeds can be retained in the soil from year to year, their recovery will be slower than recovery of aboveground plant responses.

5. Do legacies of grazing persist in soil nutrients? Grazing is known to alter concentrations and cycling of soil nutrients. I expect rapid declines in labile forms of soil nitrogen (N), nitrate and ammonium, that are more available with grazing (Ruess and McNaughton 1988), but little change in total soil N or total soil C. Generally, for N, phosphorus (P), and potassium (K), residence time in organic matter N$\approx$P$>$K (Schlesinger 1997). *H5*: I hypothesized that the persistence of soil nutrient legacies will depend on how quickly a particular nutrient cycles in the organic matter of soil. Specifically, I hypothesize that there will be no difference in labile inorganic forms of N between recovery and ungrazed areas. Similarly, K, which cycles relatively rapidly, will not be different between recovery and ungrazed areas, while N and P will show difference among treatments.

I tested these hypotheses by measuring changes in the aboveground community, belowground bud bank and seed bank, and soil nutrients in sites that were persistently grazed by cattle, sites with grazers excluded, and sites rested after removal of grazers (“recovery”).

3.2 Methods

3.2.1 Site Description

Konza Prairie Biological Station (KPBS) is a 3487 ha tallgrass prairie preserve in the Flint Hills of northeast Kansas, USA (39°05′ N, 96°35′ W). KPBS experiences a continental climate, with warm, wet summers and cool, dry winters. Mean monthly air temperatures range from -2.7 °C in January to 26.6 °C in July. Total annual precipitation averages 880 mm [1983-2012, NOAA National Climate Data Center (2013)], with 79% falling during the growing season (April to October). However, precipitation patterns in this region are characterized by high variability among years, ranging from 513 to 1435 mm annually in the period 1983-2012. Annual precipitation recorded at Konza Prairie was near or above average for 2008, 2009, and 2010 (1153 mm, 987 mm, and 842 mm), but below average for 2011, and 2012 (815 mm and 570 mm respectively), with 82, 84, 85, 64, and 74% falling during the growing season each year (Konza HQ1MET weather station). A matrix of C_4 grasses dominates the vegetation, including big bluestem (*Andropogon gerardii* Vit.), Indian grass (*Sorghastrum nutans* [L.] Nash), and little bluestem (*Schizachyrium scoparium* Michx.), with a wide variety of sub-dominant C_4 grasses, C_3 grasses, and forbs interspersed (Towne 2002). KPBS has steep topography, with shallow upland Florence cherty silt loam soil and deeper lowland Tully silt clay loam soil.

The unit of KPBS in which this study was conducted is located on the east side of KPBS (currently watersheds C3B and C3C). This unit was ungrazed during the 1970s and 1980s, and the entire unit was lightly grazed by cattle (*Bos taurus*) during the growing season in 1992 and 1993 (6.1 ha per cow/calf). In 1994, eight 4.9 ha pastures were set up on upland tallgrass prairie within this unit to compare the effects of bison (*Bison bison*) and cattle grazing, as described in detail in Towne et al. (2005). A 0.2 ha ungrazed control was associated with each grazed pasture. The four cattle pastures were grazed at 1.7 ha/au (hectares per animal unit) by yearling Angus by Hereford steers. From 1994 to 2007, grazers were placed on pastures in early May and removed in early October. These pastures have not been grazed since 2007. Only the four pastures randomly assigned to be grazed by cattle were included in this study. The remaining area of the larger unit containing these pastures continued to be grazed in a cow-calf operation at 6.1 ha/au until 1999.

In 2000 the stocking rates of the unit increased to 4.9 ha/au, and again in 2008 the stocking rate was increased to 3.2 ha/au. Areas that have been grazed continuously since 1994 will be called "grazed"; areas that have been ungrazed since 1994 will be called "ungrazed"; and areas previously grazed, but ungrazed since 2008 will be called "recovery." The grazing history of each treatment is outlined in Fig. 3.1. This unit was burned on average every two years until 1994. From 1994 until 2009, the unit was burned annually in March or April. In 2009, the unit was divided to set up a patch-burn grazing system. All sites were burned in 2009 and unburned in 2010, but only part of the sites were burned in 2011, and those unburned in 2011 were burned in 2012.

3.2.2 Plant Community Sampling

I established sixteen permanent 50 m transects at random locations in the grazed and recovery areas and sixteen 25 m transects in ungrazed areas (N = 16 transects per treatment). Bud bank sampling occurred in the dormant season (November to February) to avoid intra-annual fluctuations that result from stem recruitment and new bud formation during the growing season (Dalgleish and Hartnett 2006, 2009). To estimate bud bank density, I harvested 10×10 cm soil cores to a depth of 10 cm at stratified random locations along each transect, where sample locations were randomly selected within 5 m segments along the transect. In 2008, ten belowground samples were collected per transect. Based on preliminary analysis of 2008 data, I reduced the number of belowground samples collected per transect to eight for 2009 to 2011. All belowground samples were placed in plastic bags and stored at 4 °C pending processing. To process belowground samples, I rinsed soil to isolate the rhizomes and other belowground plant organs (no more than 3 weeks prior to examination). All belowground samples were examined by a single observer using a dissecting microscope, with roots trimmed as necessary to allow thorough examination of the belowground structures. Belowground buds were counted and assigned to species when possible using bud morphology, phyllotaxy, morphology of the attached root system, and morphology of any remaining aboveground parts (see Carter et al. 2012). Forbs that could not be confidently assigned to species using belowground morphology were pooled for all analyses. Peak stem density was estimated by counting graminoid (grass and sedge) stems and forb stems in the 10 by 10 cm area directly above each belowground sample in September before the samples were harvested. Aboveground net primary productivity (ANPP) of ungrazed areas and residual biomass of grazed areas were estimated by harvesting peak standing biomass in late-August to mid-September of 2009 to 2011 in four or five 0.125 m^2 quadrats per transect. Biomass was dried at 60 °C for $\geq$ 48 h and weighed to the nearest 0.01 g. In 2010 grass biomass was estimated using a calibrated pasture disc. Aboveground plant community composition was sampled in June and August of 2011 and 2012 by visually estimating cover of each species in five 10 m^2 circular quadrats per transect using a modified Daubenmire scale.

3.2.3 Seed Bank Sampling

I used the seedling emergence method to estimate the density and composition of seeds in the persistent soil seedbank (Thompson and Grime 1979). In addition to the sixteen 25 m ungrazed transects used for vegetation and bud bank sampling (sixteen 25 m transects = eight 50 m transects), I established another eight 50 m transects in nearby ungrazed areas so that a total of sixteen ungrazed 50 m transects were sampled. For each 50 m transect, 15 soil cores (5 cm diameter to 5 cm depth = 98 cm^3 core) were extracted at 3 m intervals between 4 April and 9 April 2012. To sample only the persistent soil seed, I removed all litter from the soil surface before extracting the soil core. All cores from a transect were pooled, homogenized, and sieved with a 4 mm sieve to remove small rocks, roots, and potential vegetative propagules (e.g. rhizomes). Samples were stored at 4 °C for two weeks until the seedling emergence trial was set up in the greenhouse. Soil was spread in a 1 cm thick layer on top of a 1.5 cm thick layer of sterile vermiculite in 28by54by6.2 cm germination trays. Four control trays were included, with a 1 cm thick layer of sterile potting soil spread over vermiculite, as above, to detect potential contamination from greenhouse sources.

The greenhouse germination trials began on 24 April 2012 and continued until 24 April 2013 (one year). Trays were placed on one meter tall benches in an environmentally controlled greenhouse and watered daily to maintain adequate soil moisture. During the first 180 days of the trial, maximum daily greenhouse temperatures averaged 30.5 °C, while minimum daily temperatures averaged 21.9 °C. During the second half of the trial, maximum daily greenhouse temperatures averaged 22.5 °C, while minimum daily temperatures averaged 16.5 °C (Fig 3.2). Trays were randomly rotated every two weeks to minimize the effects of location within the greenhouse. Soil was mixed at irregular intervals to promote germination of as many seeds as possible. Incident photosynthetically active radiation (PAR) ranged from 529 to 1285 μmol m^{-2}s^{-1}, with mean of 865 μmol m^{-2}s^{-1} on 24 April 2013. Germinating olants were identified once per week for the first seven months until seedling emergence slowed, then every two weeks until the end of the trial. Seedling identification was a two-step process to maximize the amount of information collected from the trial. Seedlings were first scored as either graminoid (Poaceae, Cyperaceae, or Juncaceae) or forb. When large enough to be identified confidently, seedlings were identified to species. If a seedling could be identified in the original germination tray, it was removed from the tray and discarded. If identification to species was not possible when the seedling was small, the seedling was re-potted and allowed to grow until a confident species identification could be assigned. After the seedling emergence trial, no further attempts were made to isolate seeds from the soil. All seedlings were scored as either graminoid or forb, but 417 of the 2709 seedlings that emerged (15.4%) died before they could be identified to species (Table 3.1).

3.2.4 Soil Nutrient Sampling

Soil nutrients were sampled in February 2013 to determine how soil nutrient levels changed in recovery areas compared to grazed and ungrazed areas. Soil was sampled systematically along the permanent 50 meter transects used for seed bank sampling. Five 5 cm diameter soil cores were extracted to 5 cm depth and combined in a composite sample. Composite soil samples were sieved with a 4 mm sieve to remove plant material and rocks. Each soil sample was then prepared and tested by the Kansas State University Soil Testing Laboratory for total nitrogen (N) and total carbon (dry combustion), ammonium-N (KCl extraction), nitrate-N (KCl extraction), phosphorus (Mehlich-3 method), and potassium (ammonium acetate extraction).

3.2.5 Statistical Analyses

I performed all data analyses with R 3.0 (R Core Team 2013). Transects were treated as the experimental unit and sub-sample measurements within transects were combined by calculating the mean response of each transect within years. The assumption of normality of response variables was checked by visual inspection of data distributions and normal quantile (Q-Q) plots. The effects of grazing treatments and years were tested with mixed model ANOVA using the *nlme* package

(Pinheiro et al. 2013). Grazing treatment, year, and their interaction were modeled as fixed effects and transects were modeled as random effects. Because some transects were burned in 2011 and others not, the effect of fire within 2011 was tested for each response, using Type III sums of squares to account for unequal numbers of burned and unburned transects. Model fit was tested by plotting fitted values against standardized residuals. *Post hoc* simple effects of grazing treatments within years were done using the *multcomp* package (Hothorn et al. 2008), with Bonferonni adjusted p-values. When grasses and sedges were combined for analyses, I referred to them as "graminoids," and herbaceous flowering plants other than graminoids were referred to as "forbs." I used a significance level of $\alpha = 0.05$, unless otherwise noted.

Demographic transitions were calculated on a density basis using bud and stem densities. The number of buds per stem is a metric often used as an index of meristem limitation in perennial grasses (Dalgleish and Hartnett 2006). I calculated the number of bud per stem by dividing the mean bud density per transect in the dormant season of year *t* by the mean stem density from the previous growing season. I also calculated the number of aboveground stems per bud, a measure of stem emergence from belowground buds. This metric was calculated by dividing the stem density from year *t* by the bud density from the previous dormant season (year *t-1*). Because bud densities were not available for the 2007-2008 dormant season, the number of stems per bud was not estimable for 2008.

Analysis of Community Composition

The effects of year and grazing treatment on bud bank (2009-2011), seed bank (post-2011), and aboveground (using canopy cover for 2011 and 2012) community structure were assessed using permutational multivariate analysis of variance on distance matrices (ADONIS) with 1000 permutations. This non-parametric technique partitions sums of squares of the multivariate data set and is a robust alternative to MANOVA or redundancy analysis (Legendre and Legendre 2012). I used both binary and quantitative versions of the Bray-Curtis dissimilarity matrix, using the `adonis` function in the *vegan* package (Oksanen et al. 2013). Because perMANOVA assumes constant dispersion among groups, we used the `betadisper` function, which is analogous to Levene's homogeneity of variance test, to test for multivariate homogeneity of group dispersions, where a group is defined as a treatment by year combination. Statistical significance of this test was assessed using a permutation test with 10000 permutations.

Differences in plant communities among sites (transects) were visualized using non-metric multidimensional scaling (NMDS) with random starts using the `metaMDS` function in the *vegan* package (Oksanen et al. 2013). I constrained the ordination to two dimensions. Species abundance scores were square root transformed and Wisconsin double standardized prior to analysis (Bray and Curtis 1957). Pairwise dissimilarity between plant communities (transects within years) was computed using binary and quantitative forms of the Bray-Curtis method, using species aboveground stem and belowground bud abundances.

I evaluated the effect of grazing treatments on diversity by calculating taxonomic species richness (S), species diversity (Shannon diversity, H'), and evenness ($H'/\ln(S)$) for the bud bank, the seed bank, and the aboveground vegetation as estimated by canopy cover. Shifts in species relative abundance in response to grazing treatment were analyzed by constructing rank-abundance diagrams using the mean relative abundance of each species per transect pooled by treatment within each year. I did not directly compare the bud bank to the seed bank or canopy cover because of differences in the scale and timing of sampling. Shifts in the relative abundance of life history strategies (annual, biennial, perennial) and growth forms (forb, graminoid, and shrub) were evaluated by calculating the relative abundance of each species in a transect within each year for the bud bank, seed bank, and canopy cover. Relative abundances were then summed by life history or growth form. Differences in relative abundance by treatment were tested using analysis of variance, with *post-hoc* multiple comparisons performed as appropriate, with Bonferonni-adjusted p-values to control the error rate ($\alpha = 0.05$).

3.3 Results

3.3.1 Bud bank density and demography

I hypothesized that when grazers were removed, bud bank density and demographic rates in recovery areas would quickly resemble the density and demography of ungrazed areas. My hypothesis was partially supported. Bud bank densities were highly variable after grazers were removed, but appeared to become similar to ungrazed areas more slowly than demographic rates. Demographic rates in recovery areas slowly became more similar to ungrazed areas over four years.

Graminoid stem densities did not differ by treatment but showed a year effect and a marginally significant treatment by year interaction (Fig. 3.3a). While treatments were not different within years, dynamics of graminoid stem densities in recovery areas were more similar to the dynamics of ungrazed areas than grazed areas. Forb stem densities showed a strong treatment by year interaction. Post-hoc analysis of treatments within year revealed that recovery areas had greater forb stem densities than ungrazed areas in the first year of recovery (2008), and greater stem densities than both grazed and ungrazed areas in the second year (2009) of recovery (Fig. 3.3c). I observed no treatment effect on forb stem density in 2010 and 2011. Both total and graminoid stem densities in recovery areas tended to reflect the stem densities of ungrazed areas. Total stem densities showed significant treatment by year and year effects, and marginally significant treatment effect (Fig. 3.3e).

The effect of treatment on graminoid bud densities was marginally significant, while the year effect and treatment by year interaction were significant (Fig. 3.3b). Graminoid bud densities were generally greater in ungrazed areas than in grazed and recovery areas from 2008 to 2010, but not in 2011. The effect of grazing treatment on forb stem densities was marginally significant, though the treatment by year interaction was significant. Recovery areas tended to have a greater density of forb buds than both grazed and ungrazed areas in the first three years of recovery (Fig. 3.3d). Total bud densities reflected trends in graminoid bud densities, with a treatment by year interaction (Fig. 3.3f). From 2008 to 2010, ungrazed areas tended to have approximately 30% greater bud densities than grazed areas. Total bud densities of recovery areas were more variable than either grazed or ungrazed areas, but tended to track the dynamics of grazed areas more than ungrazed areas.

Because some transects were burned in 2011 and others unburned, I tested the effect of fire on 2011 stem and bud densities. Graminoid stem densities were affected by fire, with significant fire effect and treatment by fire interaction driven by the increase in graminoid stem densities in recovery areas (Fig. 3.4a). Forb stem density increased in all treatments in response to fire (Fig. 3.4c).Total stem density response to fire reflected the response of graminoid stems, with treatment by fire and fire effects driven by the increase in total stems in the recovery areas that were burned (Fig. 3.4e). Graminoid, forb, and total bud densities did not respond to treatment or fire (Fig. 3.4b, d, e).

Demographic transitions estimated by the number of buds per previous growing season stem (buds per stem) and number of stems per previous dormant season bud (stems per bud) indicated that recovery areas slowly became less like grazed areas and more like ungrazed areas (Fig. 3.5). Graminoid buds per stem varied between 5 and 9 across treatments and years, while 10 to 25% of graminoid buds transitioned to aboveground stems. Both graminoid buds per stem and stems per bud varied by treatment and year, and showed treatment by year interactions that were driven by the shift in demographic rates of recovery areas. In recovery areas graminoid buds per stem resembled grazed areas in 2008, but were intermediate or more like ungrazed areas by 2011. Forb buds per stem varied between 10 and 40, at least double the number of buds per stem of graminoids, and showed a significant treatment by year interaction driven by the high number of buds per stem in ungrazed areas in 2011. Approximately 10 to 20% of forb buds transitioned to aboveground stems in 2011, but approximately only 5% in 2010 and 2011, driving a significant year effect on forb stems per bud. Like graminoids, total buds per stem showed treatment and year main effects, with a marginally significant treatment by year interaction (Fig. 3.5e). In the first year after release from grazing, buds per stem of recovery areas were indistinguishable from grazed areas. Within four years, buds per stem of recovery areas became more like ungrazed areas. Similarly, the stem-to-bud transition showed a significant treatment by year interaction, with recovery areas initially more similar to grazed areas, but with time becoming more like ungrazed areas (Fig. 3.5f).

Fire altered graminoid buds per stem in 2011 but not stems per bud (Fig. 3.6a, b). Forbs released more buds in stems in grazed and recovery areas, but had fewer buds per stem in all treatments in response to fire (Fig. 3.6c, d). Overall, recovery areas released more buds into stems in response to fire in 2011, but produced fewer buds per stem. Demographic transitions in grazed and ungrazed areas showed little response to fire (Fig. 3.6e, f).

3.3.2 Aboveground Net Primary Productivity

Peak aboveground biomass in recovery and ungrazed areas was used to estimate ANPP, while aboveground biomass from grazed areas represented residual biomass after season-long grazing by cattle, and was included as an index of grazing intensity. Graminoid biomass varied by treatment and year, with a marginally significant treatmentby year interaction (Fig. 3.7a). Residual graminoid biomass in grazed areas was 15 to 40% lower than biomass in ungrazed and recovery areas. In 2011 residual biomass was reduced in burned, grazed areas relative to unburned, grazed areas, reflecting the preference of cattle for recently burned pasture (Fig. 3.7b). Forb biomass varied by treatment and year, but was not affected by differential application of fire in 2011 (Fig. 3.7c, d). Grazed and ungrazed areas had similar forb biomass, while recovery areas tended to have greater biomass than both grazed and ungrazed areas (Fig. 3.7c). Total biomass patterns reflected patterns of graminoids, varying by treatment and year, with significantly greater biomass in recovery than grazed areas, with intermediate biomass in ungrazed areas (Fig. 3.7e). The effect of fire on total biomass also reflected the response of graminoids, with a reduction in total biomass in response to both grazing and fire (Fig. 3.7f).

3.3.3 Seed Bank Density

I did not find support for my hypothesis that seed bank density in recovery ares would be intermediate between densities of grazed and ungrazed areas. From all treatments and transects, 2709 total seedlings emerged from the soil seed bank. All seedlings were identified as either graminoid or forb, and 84.6% were identified to species (Table 3.1). Graminoid seed bank density did not vary by grazing treatment (Fig. 3.8a), with mean density of 473.9 ± 60.0 seeds per m^2 (here and below, mean ± 1 SE). Forb seed bank density varied by grazing treatment, with the lowest density in ungrazed areas (517.8 ± 94.0 seeds per m^2), and the highest density in grazed and recovery areas (1583.1 ± 260.4 and 2192.1 ± 277.6 seeds per m^2, respectively). Total seed bank density varied by grazing treatment, with the overall pattern driven by forbs (Fig. 3.8c). Ungrazed areas had the lowest seed bank density (942.2 ± 215.4 seeds per m^2), while grazed and recovery areas had the greatest seed bank density, with 2045.7 ± 325.0 and 2760.8 ± 348.0 seeds per m^2, respectively.

3.3.4 Plant Community Composition

Analysis of multivariate homogeneity was used to test if treatment by year combinations differed in their dispersion from the group centroid. The bud bank did not differ in homogeneity among treatment by year combinations ($F_{8,135}$ = 1.2, p = 0.322 and $F_{8,135}$ = 0.7, p = 0.746 for quantitative and binary Bray-Curtis dissimilarity, respectively). Multivariate homogeneity did not differ among treatments for the seed bank when the quantitative dissimilarity index was used ($F_{2,45}$ = 2.3, p = 0.121), but did differ when the binary index was used ($F_{2,45}$ = 4.9, p = 0.013). Pairwise tests indicated that dispersion in grazed and recovery areas was less than dispersion in ungrazed areas. Similarly, dispersion did not differ aboveground when the quantitative index was used ($F_{5,82}$ = 2.1, p = 0.08), but did differ when the binary index was used ($F_{5,82}$ = 4.9, p = 0.002). As in the seed bank, pairwise tests indicated that aboveground dispersion in grazed and recovery areas was less than dispersion in ungrazed areas.

While grazing treatment ($F_{2,135}$ = 5.4, $p < 0.001$, R^2 = 0.062) and year ($F_{2,135}$ = 12.4, $p <$ 0.001, R^2 = 0.142) both appeared to be important in structuring the bud bank community, we did not observe any change in recovery areas relative to grazed or ungrazed areas over time (treatment by year, $F_{4,135}$ = 0.9, p = 0.62, R^2 = 0.02). The bud bank of recovery areas was most similar to the bud bank of grazed areas for all years (Fig. 3.9a). When only the presence or absence of species in the bud bank was used in the analysis, similar patterns were observed (Treatment: $F_{2,135}$ = 13.1, $p < 0.001$, R^2 = 0.142; Year: $F_{2,135}$ = 9.9, $p < 0.001$, R^2 = 0.108; Treatment by Year: $F_{4,135}$ = 0.8, p = 0.732, R^2 = 0.016; Fig. 3.9b).

Although visual inspection of the NMDS ordination suggested little to no difference in seed bank composition among grazing treatments (Fig. 3.9c, d), treatment did affect the composition of the seed bank (quantitative: $F_{2,45}$ = 4.3, $p < 0.001$, R^2 = 0.162; binary: $F_{2,45}$ = 3.0, $p < 0.001$, R^2 = 0.119).

Aboveground, community composition varied by both treatment and year for both quantitative ($F_{2,82}$ = 8.5, $p < 0.001$, R^2 = 0.143; $F_{1,82}$ = 17.3, $p < 0.001$, R^2 = 0.144) and binary ($F_{2,82}$ = 7.4, $p < 0.001$, R^2 = 0.132; $F_{1,82}$ = 14.8, $p < 0.001$, R^2 = 0.132) dissimilarity metrics. Visual inspection of the NMDS ordination revealed that recovery areas tended to group with ungrazed areas, particularly when abundance is accounted for in dissimilarity calculations. Treatment and year did not interact to affect community composition (quantitative: $F_{2,82} = 1.6$, $p = 0.061$, $R^2 = 0.027$; binary: $F_{2,82} = 0.4$, $p = 0.994$, $R^2 = 0.007$).

3.3.5 Plant Community Diversity

Species richness (S) reflected overall patterns of plant community composition. Bud bank species richness varied by treatment ($p < 0.001$), with grazed and recovery areas containing two or more species per 0.01 m^2 compared to ungrazed areas (Fig. 3.10a). Similarly, seed bank richness varied by treatment, with grazed and recovery areas more species-rich than ungrazed areas (Fig. 3.10b). Finally, species richness in the aboveground plant community varied with both treatment ($p < 0.001$) and year ($p = 0.012$), with richness the greatest in grazed sites, least in ungrazed sites, and intermediate in recovery areas. Richness was slightly greater for all treatments in 2012 compared to 2011 ($p = 0.012$, Fig. 3.10c).

I used the Shannon diversity index (H') because of its sensitivity to rare species. Diversity patterns generally reflected species richness patterns. In the bud bank, diversity varied by treatment and year ($p < 0.001$ and $p = 0.005$, respectively), and was greatest in grazed and recovery areas relative to ungrazed areas (Fig. 3.11a). Seed bank diversity also varied by treatment (marginally significant, $p = 0.09$), with ungrazed areas again relatively low compared to recovery and grazed sites (Fig. 3.11b). Aboveground, diversity varied by treatment and year ($p < 0.001$), and was greatest in grazed sites, least in ungrazed sites, and intermediate in recovery sites (Fig. 3.11c).

I used Shannon diversity divided by the natural log of richness (S) as the index of evenness. In the bud bank, evenness varied by year ($p = 0.018$) but not by treatment (Fig. 3.12a). Seed bank evenness varied by treatment ($p = 0.001$), with evenness low in recovery areas relative to grazed and ungrazed areas (Fig. 3.12b). Aboveground plant community evenness showed a significant treatment by year interaction, with evenness generally lower in 2012 compared to 2011. Evenness was greatest in grazed areas, least in ungrazed areas, and intermediate in recovery areas (Fig. 3.12c).

Rank-abundance diagrams confirmed the observed patterns in species richness and evenness. In the bud bank, the steep slope of the ungrazed areas indicated low evenness, while grazed and recovery areas had similar, higher evenness relative to grazed areas. Grazed and ungrazed areas had lower species richness than recovery areas (Fig. 3.13a–c). In the seed bank, all treatments appeared to have similar evenness, while recovery areas had greater species richness than grazed or ungrazed areas (Fig. 3.13d). Aboveground, grazed areas were more even and species rich than ungrazed and recovery areas. Recovery areas had intermediate species richness relative to grazed and ungrazed areas (Fig. 3.13e, f).

3.3.6 Relative Abundance of Life Histories and Growth Forms

While perennial species were the only life history strategy present in the bud bank (data not shown), bud abundance did vary by growth form (Fig. 3.14). Graminoids, the most abundant growth form, did not vary in abundance by treatment ($F_{2,45} = 0.6$, $p = 0.539$), but varied by year ($F_{2,90} = 5.4$, $p = 0.006$), with a marginally significant treatment by year interaction ($F_{4,90} = 2.2$, $p = 0.06$). Similarly, forbs, the second most abundant growth form, did not vary in relative abundance by treatment ($F_{2,45} = 0.5$, $p = 0.586$), but varied by year ($F_{2,90} = 7.5$, $p = 0.001$), with a marginally significant treatment by year interaction ($F_{4,90} = 2.4$, $p = 0.057$). The only species of shrub observed in the study was *Amorpha canescens* L., and it accounted for a very small fraction of the total buds. Treatment and treatment by year effects were not significant ($F_{2,45} = 0.5$, $p = 0.608$; $F_{4,90} = 0.1$, $p = 0.97$; respectively), but shrub bud abundance varied by year ($F_{2,90} = 4.9$, $p = 0.009$).

Perennial, biennial, and annual life histories were represented in the seed bank. Because only one biennial species (*Verbascum blatteria* L.) was present, and only on two transects, I excluded biennials from statistical analysis of life history abundance. Recovery and grazed sites did not differ in the relative abundance of annuals and perennials, with each life history strategy represented by approximately 50% of the individuals. However, in ungrazed areas perennials composed approximately 70% of the seed bank and annuals approximately 30% (Fig. 3.15a). Forb, graminoid, shrub, and tree growth forms were encountered in the seed bank. Because only 15 total shrub or tree germinants were observed from all transects (Table 3.1), trees and shrubs were excluded from statistical analysis of growth form abundance. In both grazed and recovery sites, forbs composed 75 to 80% of the seed bank, and graminoids composed 20 to25%. In ungrazed areas, however, graminoids and forbs were equally represented in the seed bank (Fig. 3.15b). The changes in both life history and growth form were driven by decreased relative abundance of annual forbs and increased relative abundance of perennial graminoids in ungrazed areas (Fig. 3.15c).

There was no difference in aboveground relative abundance of life history strategies or growth forms between years ($p \approx 1$) using canopy cover to estimate abundance, so data were pooled across years (2011 and 2012). Because perennials clearly dominated aboveground, with no difference among treatments (Fig 3.16a), analysis of variance was not performed to determine the effect of treatment on relative abundance of life history strategies. Due to the treatment by growth form interaction, six multiple comparisons were performed to determine the effect of treatment within graminoid and shrub growth forms. No difference in forb relative abundance was observed among treatments; grazed sites had lower relative abundance of graminoids than recovery or ungrazed sites. Shrubs tended to have the greatest relative abundance at grazed sites and the lowest relative abundance on ungrazed sites (Fig. 3.16b).

3.3.7 Soil Nutrients

I analyzed the effect of both grazing treatment and previous season fire on soil nutrients. Phosphorus showed a treatment by fire interaction ($p = 0.024$), with no treatment effect in unburned areas, but a reduction in phosphorus in recovery areas relative to grazed and ungrazed areas. Overall, unburned areas had lower soil phosphorus than burned areas (Fig. 3.17a). Potassium and NH_4-N showed no variation by fire or treatment (Fig. 3.17b, c). Nitrate-N varied by treatment ($p = 0.007$), with grazed areas generally having the highest concentration and ungrazed areas the lowest (Fig. 3.17d). Total nitrogen showed a treatment by fire interaction ($p = 0.002$). In unburned areas, total nitrogen was greatest in grazed areas and lowest in ungrazed, while in burned areas, total nitrogen in recovery areas was low relative to grazed and ungrazed areas (Fig. 3.17e). Total C varied by treatment but not fire, where total C tended to be greatest in grazed areas compared to both recovery and ungrazed areas. The C:N ratio showed a significant treatment by fire interaction ($p = 0.002$, Fig. 3.17f). When unburned, the C:N ratio was lowest in grazed areas and highest in ungrazed areas, with recovery areas intermediate. When burned, the C:N ratio was lowest in ungrazed areas and greatest in recovery areas, with grazed areas intermediate (Fig. 3.17g). The nitrogen to phosphorus (N:P) ratio did not vary by treatment but did vary in response to fire, with lower N:P ratio in burned areas relative to unburned areas (Fig. 3.17h).

3.4 Discussion

I found that after grazers were removed, aboveground plant community diversity, stem density, and productivity in recovery areas quickly became similar to ungrazed areas. In contrast, belowground bud bank density, composition, and demography continued to resemble grazed areas more than ungrazed areas for several years. Similarly, after four years without grazing, the soil seed bank still resembled grazed areas more than ungrazed areas. Soil nutrients in recovery areas did not consistently resemble grazed or ungrazed areas, and the response depended on recent burning history.

3.4.1 Changes in Bud Bank Density and Demography

I hypothesized (*H1*) that without grazers to alter key environmental factors and processes, bud bank density and demographic rates in recovery areas will quickly resemble the density and rates of ungrazed areas. I found partial support for this hypothesis. Bud bank densities did not quickly return to the ungrazed state when grazers were removed, but bud demographic rates in recovery areas gradually become more similar to ungrazed areas and less similar to grazed areas over the four years of this study.

Like Dalgleish and Hartnett (2009), I found that grazing did affect bud bank density and demography. Grazed areas generally had higher stem densities but produced fewer buds per stem than ungrazed areas (5 to 7 vs. 6.5 to 10 buds per stem). As expected, a greater proportion of buds in grazed areas grew out into aboveground stems than in ungrazed areas (15 to 25% vs. 8 to 20% of buds, respectively). Thus, grazing affects both the production of new buds and the outgrowth of buds into stems. Dalgleish and Hartnett (2009) also found increased rates of stem emergence from the bud bank and reduced bud bank densities in grazed areas. I hypothesized that because bud bank demography is largely determined by aboveground conditions, it would quickly return to the ungrazed state when grazers were removed. My hypothesis was partly supported, as I generally found total buds per stem and stems per bud returned to ungrazed levels within four years of removing grazers. This lag in bud bank demography may reflect the two to three year residence time of C_4 grass buds in the bud bank (Hendrickson and Briske 1997, Ott and Hartnett 2012).

Reduced bud bank densities in grazed areas did not appear to affect the resilience of ANPP after grazers were removed. Total bud densities in recovery sites initially tended to be less than bud densities in ungrazed areas, but ANPP in recovery sites at least equal to ANPP in ungrazed sites. This suggests that while bud bank density may help explain variation in ANPP across broad spatial scales (Dalgleish and Hartnett 2006, 2009), effects of resource availability (including soil nutrients) on plant growth may be more important factors determining grassland productivity.

I also found that fire had a relatively small effect on graminoid bud demography in 2011, which may have resulted from below-average growing season precipitation (64% of 815 mm received in 2011). In contrast, more forb stems grew from belowground buds in response to fire, but forb stems in burned areas generally had fewer buds per stem. This suggests that while fire facilitates increased forb stem density, competition from grasses may limit increased bud production by these stems.

3.4.2 Seed Bank Density

Since large ungulate grazers preferentially feed on graminoids and avoid forbs in tallgrass prairie (Hartnett et al. 1996), I hypothesized (*H2*) that because rest from grazing would result in greater competitive dominance of grasses, forb seed input into the seed bank and overall seed bank density would decline as time since grazing increased. Furthermore, because disturbance from fire and grazing may be more unpredictable in grazed areas, I expected a greater proportion of species in the plant community of grazed areas to produce seeds that remained viable in the soil for long periods (Harper 1977). I found partial support for this hypothesis. Grazed areas had greater seed bank density than ungrazed areas, but I did not observe a decline in seed bank density after grazers were removed. Seed bank densities of recovery areas were at least as great as the seed bank densities in grazed areas.

In contrast to our results, a previous study in the northern Great Plains of North America found that after more than 35 years of consistent grazing, seed bank density peaked in lightly grazed areas, with densities in the top 6 cm of soil of 5096 seeds m^{-2} in ungrazed areas, 6014 seeds m^{-2}

in lightly grazed areas, and 4420 seeds m^{-2} in heavily grazed areas (Willms and Quinton 1995). Cooper (2006) found that reindeer grazing in the Arctic reduced soil seed bank density by half (596 seedlings emerged m^{-2} in ungrazed versus 263 seedlings emerged m^{-2} in grazed). In African savanna, O'Connor and Pickett (1992) studied two sites and found that the seed bank density in litter and the top 2 cm of soil was greater for lightly grazed (4320 and 908 seeds m^{-2}) relative to heavily grazed areas (2832 and 430 seeds m^{-2}), and composed mostly of seeds of perennial grasses that did not contribute to the persistent soil seed bank. Rabinowitz (1981) examined the seed bank in the top 30 cm of soil in ungrazed tallgrass prairie in Missouri, USA. She estimated the seed bank density to be 6470 seeds m^{-2}, composed of 70% graminoids and 30% forbs, with the majority of the seeds from the graminoid *Juncus tenuis* Willd. Abrams (1988) previously studied ungrazed tallgrass prairie at Konza Prairie in northeast Kansas, USA, and found that unburned prairie had higher seed bank density in the top 5 cm of soil (2892 seeds m^{-2}) than prairie burned every four years (2430 seeds m^{-2}) or prairie burned annually (1453 seeds m^{-2}), with the difference driven primarily by increased density of the graminoids *Poa pratensis* L. and *Sporobolus cryptandrus* (Torr.) A. Gray in unburned areas.

My estimate of total seed bank density in ungrazed, annually burned areas was 942 seeds m^{-2} and corresponded to the estimate of Abrams (1988) of 1453 seeds m^{-2} under similar management. I found that the greater seed bank density in grazed and recovery areas was driven primarily by greater forb seed density. In contrast, Loydi et al. (2012) found that grazing by feral horses in Argentinian grassland reduced total seed bank density relative to ungrazed areas, primarily by reducing the density of graminoid seeds. The increased forb seed bank density I observed could result from increased production of seed; increased incorporation of seeds into the seed bank; or decreased loss from the soil seed bank from mortality, predation, or recruitment of seeds. Although my study design did not directly test the different mechanisms that could cause changes in seed bank density, I observed increased forb stem densities and forb aboveground biomass relative to grazed and ungrazed areas in the years immediately following release from grazing. Increased production of seed may have accompanied the increased stem density and biomass of forbs. Furthermore, with reduced soil disturbance, increased competition for light, and change in light quality at the soil surface, fewer seeds may have been recruited from the seed bank as sites recovered from long-term grazing.

The seed bank of grazed and recovery areas was not only more dense, with relatively more forbs than ungrazed sites, but I also observed a shift in the relative abundance of annual and perennial species. Areas with unpredictable disturbance are expected to have greater relative abundance of plant species with long-lived seeds, which tend to be annual species (Harper 1977). I found that grazed and recovery areas, which had the least predictable disturbance regime for the past 20 years had a greater abundance of annual species not only aboveground, but also in the seed bank. Since long-lived or clonal species often produce seeds that do not persist in the soil seed bank (Baskin and Baskin 1998), the composition of the seed bank is often dissimilar to the aboveground vegetation in tallgrass prairie (Abrams 1988, Rabinowitz 1981). However, I found that the relative abundance of annual species was lower both above- and belowground in long-term ungrazed areas relative to areas currently or recently grazed. The *relative* similarity of growth forms in the seed bank and the aboveground plant community increased the longer a particular disturbance regime persisted.

Managing for a high density, diverse soil seed bank could increase grassland stability by enhancing the ability of plant community to resist invasion by non-resident species, including undesirable rangeland weeds such as *Bromus tectorum* L. With high-density seed banks of resident plant species, native seedlings may be able to colonize canopy gaps and pre-empt resources before non-resident species (Davis et al. 2000). A diverse seed bank may increase the diversity of germination cues and phenologies represented in the seed bank, limiting the spatial and temporal opportunities for non-resident establishment. Previous studies have shown that plant community response to disturbance may be predicted by composition of the soil seed bank (Marks and Mohler 1985, Van Der Valk and Verhoeven 1988), that managing the soil seed bank may be an important aspect of protecting rare species (Keddy and Reznicek 1982), and that seed banks may reflect rangeland quality (Willms and Quinton 1995). In addition to other benefits (Schuler et al. 2006), patch-burn grazing management may be a good approach for establishing or maintaining a high-density, diverse soil seed bank in native and restored grassland plant communities because it incorporates diverse burning and grazing regimes across years, discouraging multi-year dominance by one functional group.

3.4.3 Bud bank, seed bank, and aboveground recovery from grazing

I hypothesized (*H4*) that because buds and seeds are long-lived and can be retained in the soil from year to year, their return to an ungrazed state will be slower than recovery of aboveground plant responses. I found support for this hypothesis: bud and seed banks in recovery areas remained similar to grazed areas or slowly changed over time, but stem density and ANPP in recovery areas was indistinguishable from ungrazed areas within one growing season.

This study demonstrates short-lived aboveground effects of grazing and longer-term belowground impacts of grazing in the bud and seed banks. Each of these parts of the plant community plays a different role in plant community and population responses to and recovery from disturbance. Because tallgrass prairie and other grasslands are herbaceous plant communities that regenerate annually aboveground, the aboveground portion of the plant community represents emergence from belowground reservoirs of propagules (bud and seed banks). Emergence from these belowground reservoirs depends on the effects of disturbance regime on microclimate conditions and biotic interactions (Arrieta and Suarez 2005, Benson and Hartnett 2006, Tozer and Auld 2006). These environmental conditions, including light, temperature, and nutrients, are known to differentially affect emergence of species from the bud and seed banks (Baskin and Baskin 1998, Butler and Briske 1988, Tomlinson and O'Connor 2004, Williamson et al. 2012). Thus the species detected aboveground, and their relative abundance, are determined both by the presence and abundance of species in the bud and seed banks and by successful emergence from these reservoirs.

I found support for my hypothesis that the aboveground plant community in recovery areas would more quickly become like ungrazed than the belowground bud bank plant community. Measures of the aboveground plant community in recovery areas, including stem density and productivity, were indistinguishable from ungrazed areas within one growing season, while aboveground plant community composition resembles long-term ungrazed areas within five years. The annual fluctuations in total stem density in recovery areas match the fluctuation in ungrazed areas, which had lower stem densities than grazed areas in three of the four years of this study. Similarly, productivity in recovery areas was at least equal to productivity in ungrazed areas in all years. The ordinations of aboveground canopy cover show that plant community composition of the recovery plots is more like the ungrazed sites than the grazed sites in 2011 and 2012, 4 to 5 years after grazers were removed. Belowground in the bud bank, however, the recovery areas in many ways continue to resemble grazed areas. Both total bud bank densities and bud bank composition in recovery areas tend to resemble grazed areas more than those of ungrazed areas.

Although somewhat buffered from short-term variation in productivity and reproduction of the aboveground plant community, long-term maintenance of the bud bank depends on continued growth aboveground, while replenishing the seed bank depends on successful production of seed by sexual or asexual means. The dominant grasses, which compose the majority of the bud bank in tallgrass prairie, turn over the most of their buds within two years, but some have buds that last three or more years (Ott and Hartnett 2012, Hendrickson and Briske 1997). Production of new buds is largely insensitive to short-term grazing and drought (Hendrickson and Briske 1997, N'Guessan and Hartnett 2011, Chapter 2). The longevity of buds and their continued production through disturbance contribute to the stability of plant community productivity and species composition.

I found support for my hypothesis (*H3*) that changes in diversity during recovery would be driven by changes in relative abundance of species in both the bud and seed banks, with most species persisting in the plant community due to their long-lived propagules. While species were quickly lost aboveground, species richness and rank abundance remained relatively unchanged belowground in the seed and bud banks. Plant species vary in the longevity of their seeds in the soil seed bank, and longevity is often correlated with both the dominant regeneration strategy of a species and the preferred disturbance regime of a species (Thompson and Grime 1979, Thompson et al. 1993, Wijayratne and Pyke 2012). This study confirms the results of other studies of seed banks in tallgrass prairie, demonstrating that the dominant grasses in tallgrass prairie have a very large bud bank, but their seeds are not viable for more than one year in the soil (e.g. Abrams 1988, Rabinowitz 1981). Many of the forbs and sub-dominant grasses, however, do form long-term soil seed banks, and often rely on unpredictable aboveground disturbances to create opportunities to recruit and establish successfully (Davis et al. 2000).

Many studies have noted that ungulate grazing is necessary to maximize diversity in tallgrass prairie and other grasslands (e.g. Collins and Calabrese 2012, Hartnett et al. 1996), but it is not always possible or desirable to continuously graze grasslands. I found that species richness and Shannon diversity remain high belowground in the bud and seed bank and somewhat elevated aboveground for at least 5 years after grazers are removed. The many changes that accompany grazing, including removal of aboveground biomass, changes in nutrient cycling, increased light availability, and increased soil disturbance affect plant population dynamics, leaving reservoirs of long-lived propagules belowground that have shifted in density, diversity, and overall composition. I demonstrated that these propagule banks contribute to a diverse plant community during short-term rest from grazing.

Changes in overall community composition for the bud bank, seed bank, and aboveground may reflect the rate at which individuals turn over in these parts of the plant community. The bud bank of recovery areas may remain more similar to the bud bank of grazed areas because rhizomes and other perennating organs remain viable for several years in the soil. On the contrary, aboveground plant community composition may quickly become more like ungrazed areas because aboveground stems die and are replaced by new stems annually. The seed bank, which has individuals with greatest longevity, may be unlike both grazed and ungrazed areas because it integrates seeds from multiple years that were produced under various conditions. Therefore, I found that grazed and ungrazed sites may remain dissimilar belowground for many years because of persistence of long-lived seeds and buds in the soil.

3.4.4 Soil Nutrient Legacies

I hypothesized (*H5*) that the persistence of soil nutrient legacies would depend on how quickly a particular nutrient cycles in the soil organic matter. I found some support for this hypothesis. As expected, I found no difference in labile inorganic forms of N in the soil between recovery and ungrazed areas. Potassium did not vary by treatment or fire, suggesting that these disturbances do not have a large effect on cycling of this nutrient. Total N and P, which have similar residence times in organic matter across a variety of ecosystems (Schlesinger 1997), showed similar response to grazing treatments, but different responses to fire. Total C in recovery areas also appeared to decline after grazers were removed.

In tallgrass prairie, grazing is known to affect rates of nutrient cycling (Knapp et al. 1999). Similarly, fire affects nutrient cycling. Nutrients that are easily volatilized by fire, most importantly nitrogen, may decline in availability with frequent burning, while phosphorus and potassium, which remain in the ash in mineral form (Eisele et al. 1989), are not lost and may briefly be more available to plants following fire. As other studies have reported (Eisele et al. 1989), I found that plant-available phosphorus is low in unburned prairie relative to burned prairie. However, when burned, phosphorus concentrations were depressed in recovery areas relative to grazed and ungrazed areas, a trend that is mirrored by total nitrogen. This suggests the effects of grazing and fire on plant-available phosphorus are linked to their effects on total nitrogen.

Grazers consume recalcitrant forms of nitrogen in plants and excrete nitrogen in more labile forms (Ruess and McNaughton 1988), increasing rates of nitrogen cycling in grazed areas. Grazers may reduce loss of nitrogen to fire by increasing the patchiness of fire and the reducing the amount of biomass remaining to be consumed by fire (Hobbs et al. 1991). In contrast, Biondini et al. (1998) found that grazing decreased nitrogen mineralization and increased loss of soil N in northern mixed grass prairie of North America. In south African savanna, Coetsee et al. (2011) found higher foliar nitrogen on grazing lawns, but did not find greater total soil nitrogen. I found that when sites were unburned the previous year, grazed areas had the highest total soil nitrogen, while recovery areas were not different from ungrazed areas. This suggests that grazing decreases nitrogen loss from tallgrass prairie, but that additional nitrogen is quickly lost after grazers are removed. However, when sites were burned the previous year, recovery areas had low total soil nitrogen relative to both grazed and ungrazed areas. Inorganic forms of nitrogen were present in relatively high concentrations in the soil across all treatments. These high levels perhaps reflect both the timing of sampling, in early spring when inorganic nitrogen levels are the highest (Blair 1997, Knapp and Hulbert 1986), and the accumulation of inorganic soil nitrogen after two years of drought (Seastedt and Knapp 1993).

Previous studies have documented that environmental conditions determine whether grazing increases or decreases soil carbon storage (e.g. Frank et al. 1995, McSherry and Ritchie 2013, Ziter and MacDougall 2013), although overgrazing may generally lead to losses in soil carbon (Conant and Paustian 2002). Grazing may increase carbon storage by slowing mineralization of soil organic matter under some circumstances (Shahzad et al. 2012). I found that grazing increased carbon storage in tallgrass prairie, but my results suggest that this increased carbon storage is transient, with recovery sites indistinguishable from ungrazed sites six years after grazers were removed. Pineiro et al. (2010) suggest that the primary controls over soil organic carbon under grazed conditions are changes in net primary production, nitrogen stocks, and organic matter decomposition, with the relative importance of each varying with site-specific conditions, such as precipitation, temperature, and soil texture (McSherry and Ritchie 2013).

The C:N and N:P ratios indicate relative nutrient limitation in soils. When unburned the previous year, I found the lowest C:N ratio (least N limitation) in grazed areas and the greatest C:N ratio (most N limitation) in ungrazed areas, and intermediate N limitation in recovery areas. However, when burned the previous year, recovery areas had the highest C:N ratio, suggesting that sites recovering from grazing may lose nitrogen at a higher rate when burned than grazed or ungrazed areas. Unlike the C:N ratio, I observed no effect of grazing treatment on the N:P ratio, only an effect of fire, with a higher N:P ratio in unburned areas than in burned areas.

3.5 Conclusions

Grazing affects grassland plant communities and nutrient cycling long after grazers are removed because of persistent grazing effects belowground. Legacies of grazing persist in the soil, in the bud bank, and in the seed bank, even if these legacies are not immediately obvious aboveground. The rate of change and path back to the ungrazed state vary and are possibly determined by the previous grazing regime (intensity, seasonality, and longevity) and the turnover rate for each population or process.

In many cases, the legacy effects of grazing are desirable. Grazing suppresses competitive dominance of grasses and increases spatial heterogeneity relative to ungrazed prairie, enhancing plant species diversity. Increased seed bank density in grazed and recovery areas may allow plant communities to respond quickly to future soil disturbance, thus increasing overall plant community stability and resistance to invasion. Similarly, perennial plant species that were able to establish during periods of grazing are able to persist when grazers are removed, largely due to the presence of a belowground reserve of rhizomes and their associated buds. Greater availability of nutrients under grazing may permit rapid recovery of ANPP immediately after grazers are removed.

However, legacy effects of grazing can also be undesirable. For example, if non-native plant species establish, or woody plants encroach, as a result of grazing, bud and seed banks of those undesirable species linger in the soil for many years, preventing successful restoration of native grassland plant communities. Land managers will need to consider persistent effects of grazing on soil nutrients, seed banks, bud banks, and the aboveground plant community while considering various restoration approaches.

Future research should investigate if the legacy effects of grazing and recovery dynamics observed in tallgrass prairie can be generalized to other grassland plant communities. Differences in precipitation amount and timing, temperature, and soils may affect plant community response to and recovery from grazing. Relative rates of recovery for the seed bank, bud bank, and aboveground plant community may be similar across different systems because of common underlying plant population processes.

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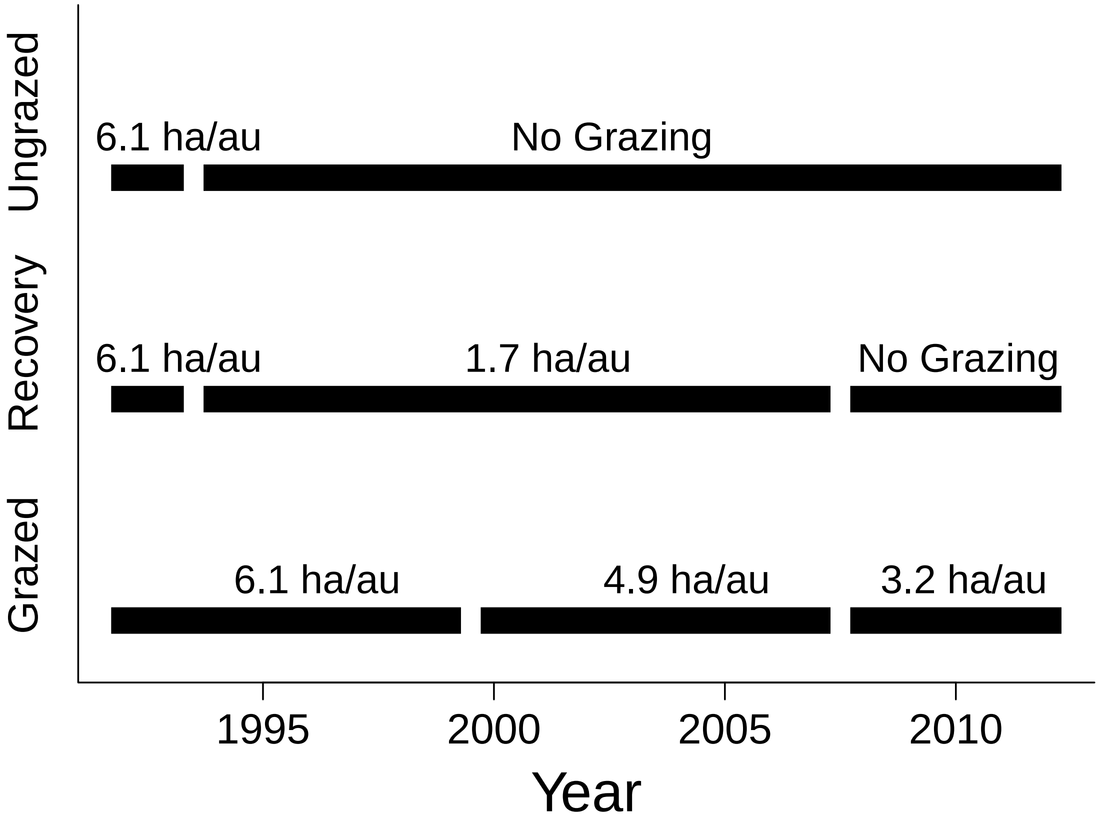

Figure 3.1: Grazing history from 1992 to 2012 for the grazing treatments applied in this study. Units refer to cattle stocking rates, where “ha/au” indicates hectares per animal unit.

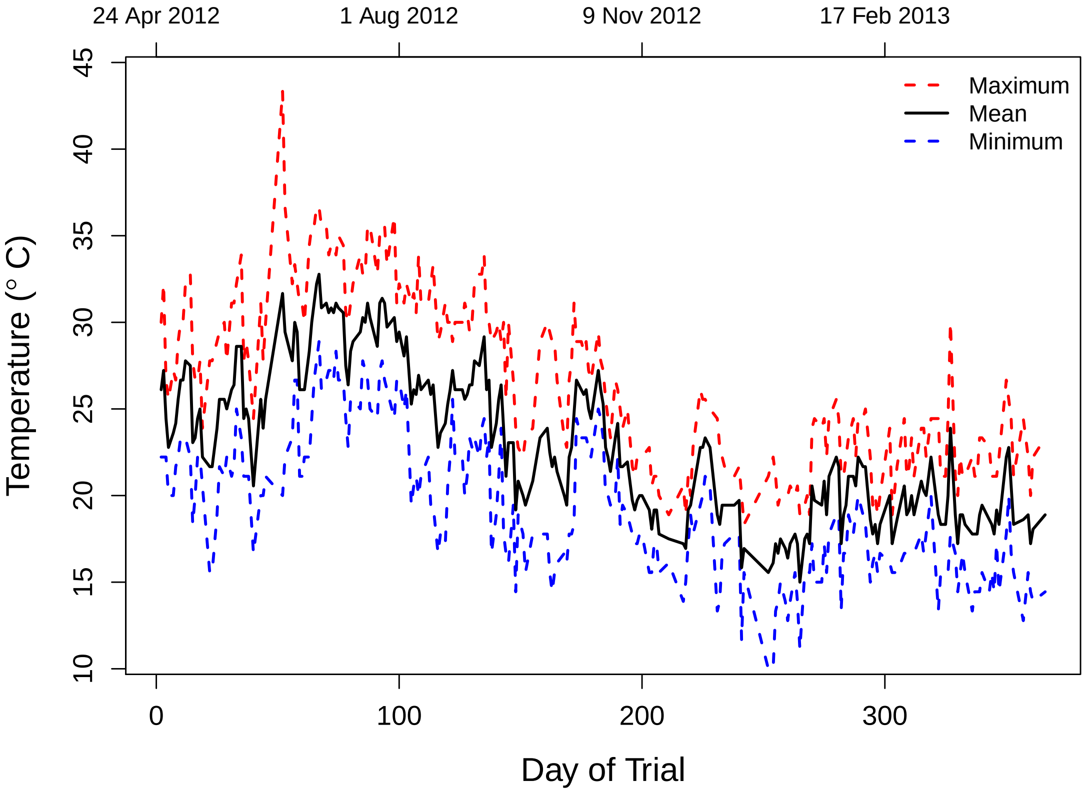

Figure 3.2: Maximum, mean, and minimum daily greenhouse temperatures during the seed bank emergence trial. Mean daily temperature is the average of the maximum and minimum temperatures. The temperature spike on 14 June 2012 was due to a blown breaker, and cooling systems were restored within 6 hours.

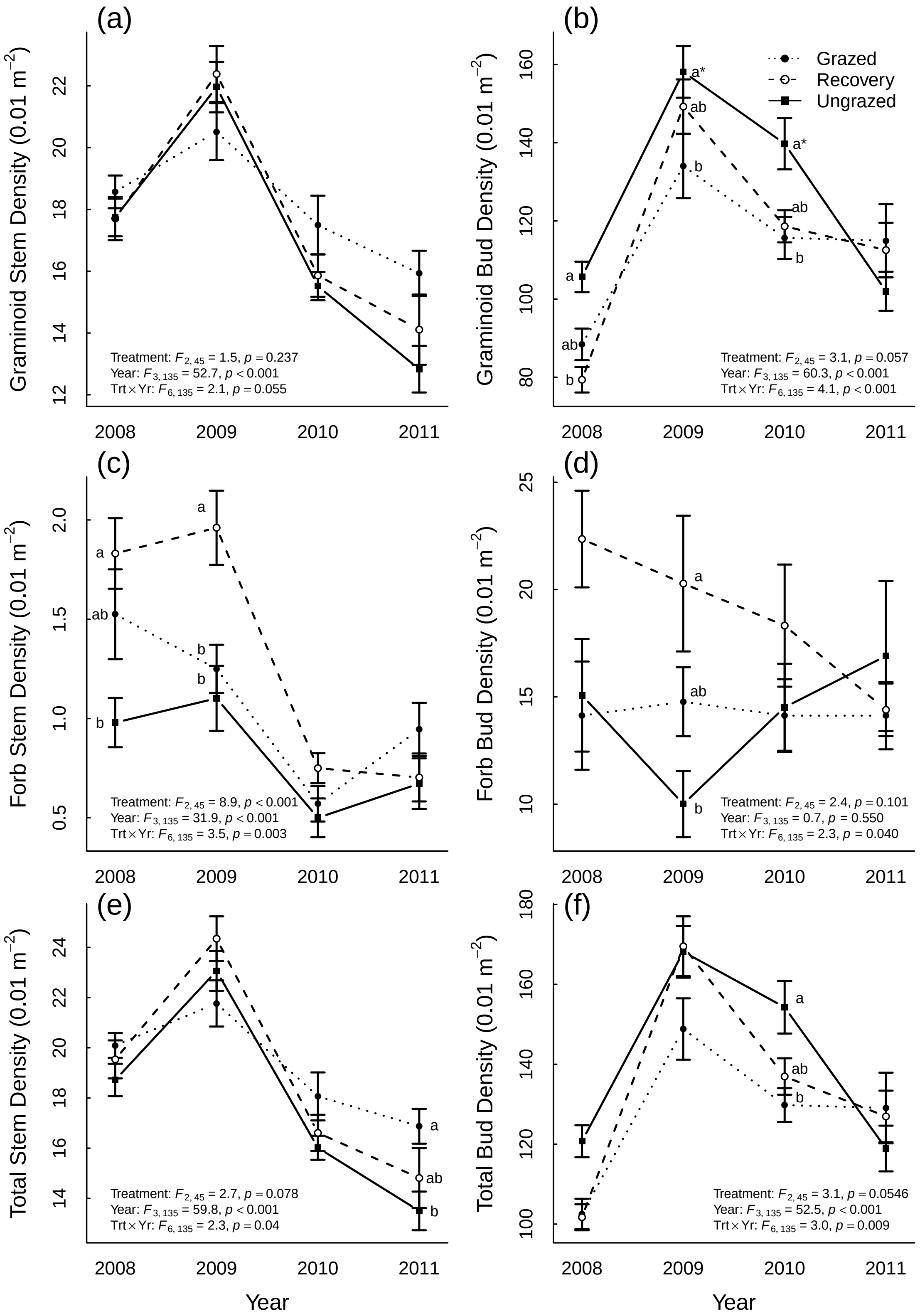

Figure 3.3: Stem (a, c, e) and bud (b, d, f) densities for graminoids, forbs, and total from 2008 to 2011. Lowercase letters indicate significant differences within years. Points are means ± 1 SE.

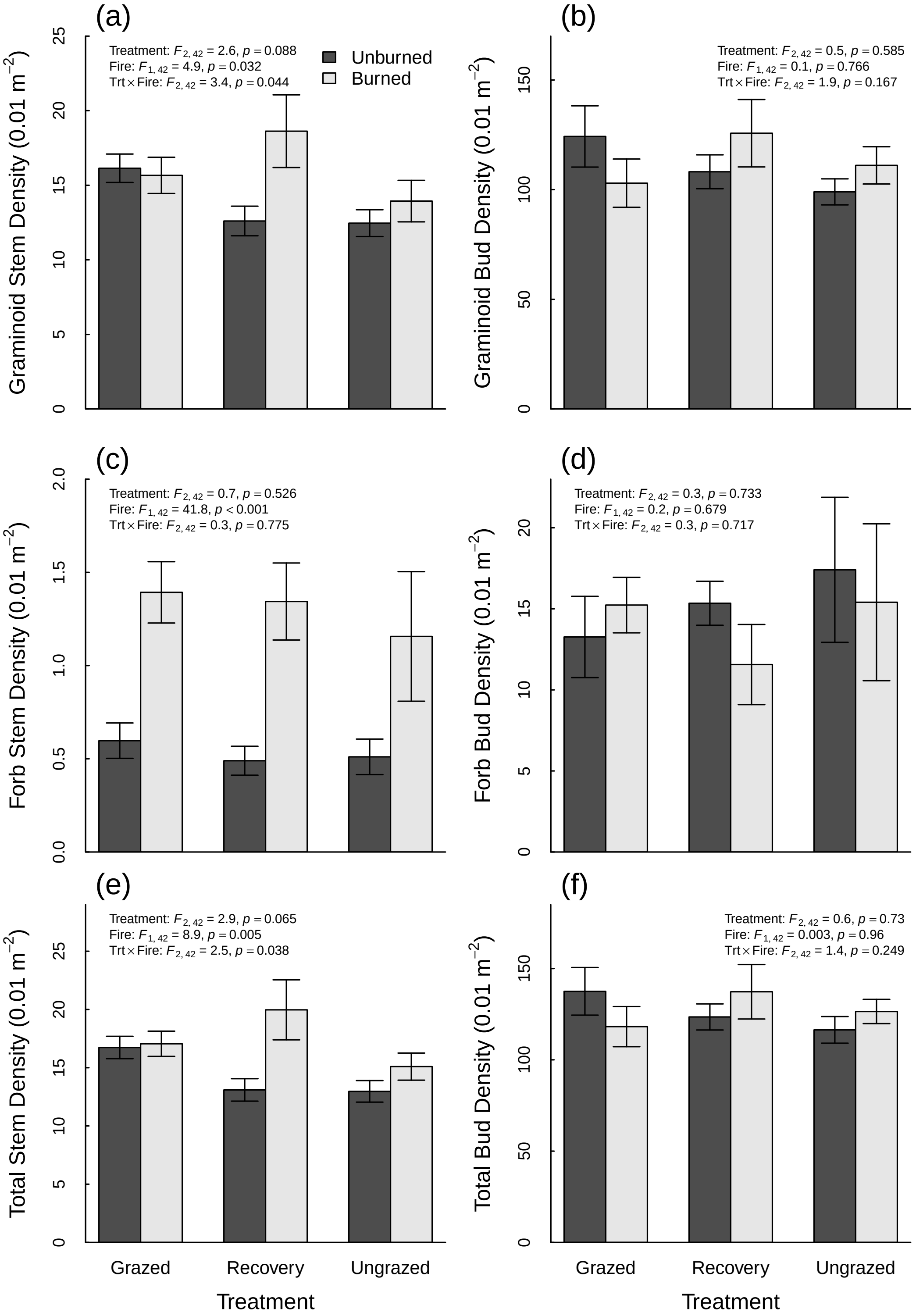

Figure 3.4: Stem (a, c, e) and bud (b, d, f) densities for graminoids, forbs, and total in 2011, showing burned and unburned areas separately. In (c), denominator degrees of freedom for year and treatment by year are 133 because two transects had no forb stems in 2010, so buds per stem was undefined. In (d), denominator degrees of freedom for treatment and year are 89 because one transect had no forb buds in 2010, so stems per bud was undefined. Bars are means ± 1 SE.

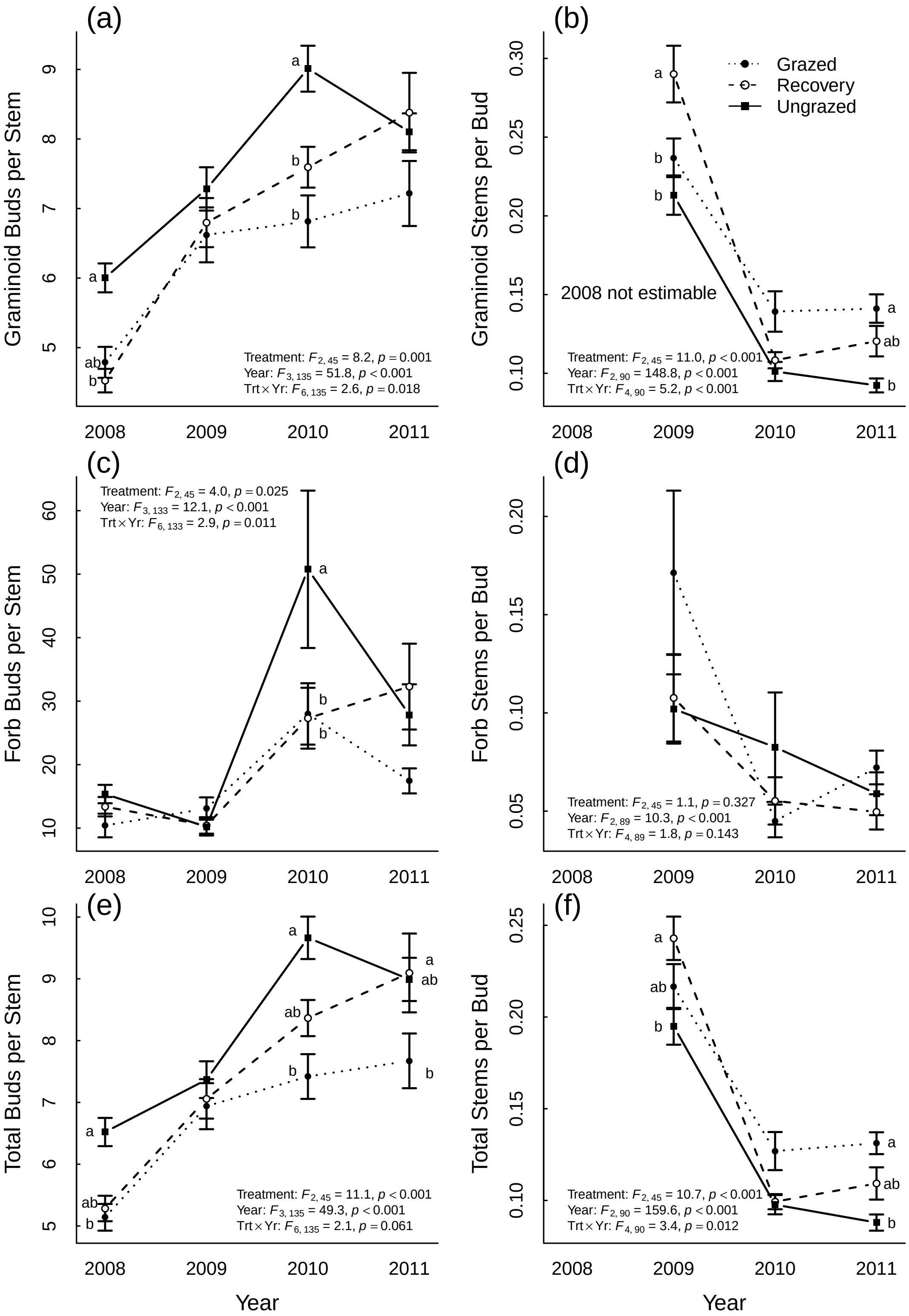

Figure 3.5: Bud bank demographic transitions from 2008 to 2011. Panels (a), (c), and (e) show transition rates from bud to stem, and panels (b), (d), and (f) show rates of bud natality by stems. Lowercase letters indicate significant differences within years. Points are means ± 1 SE.

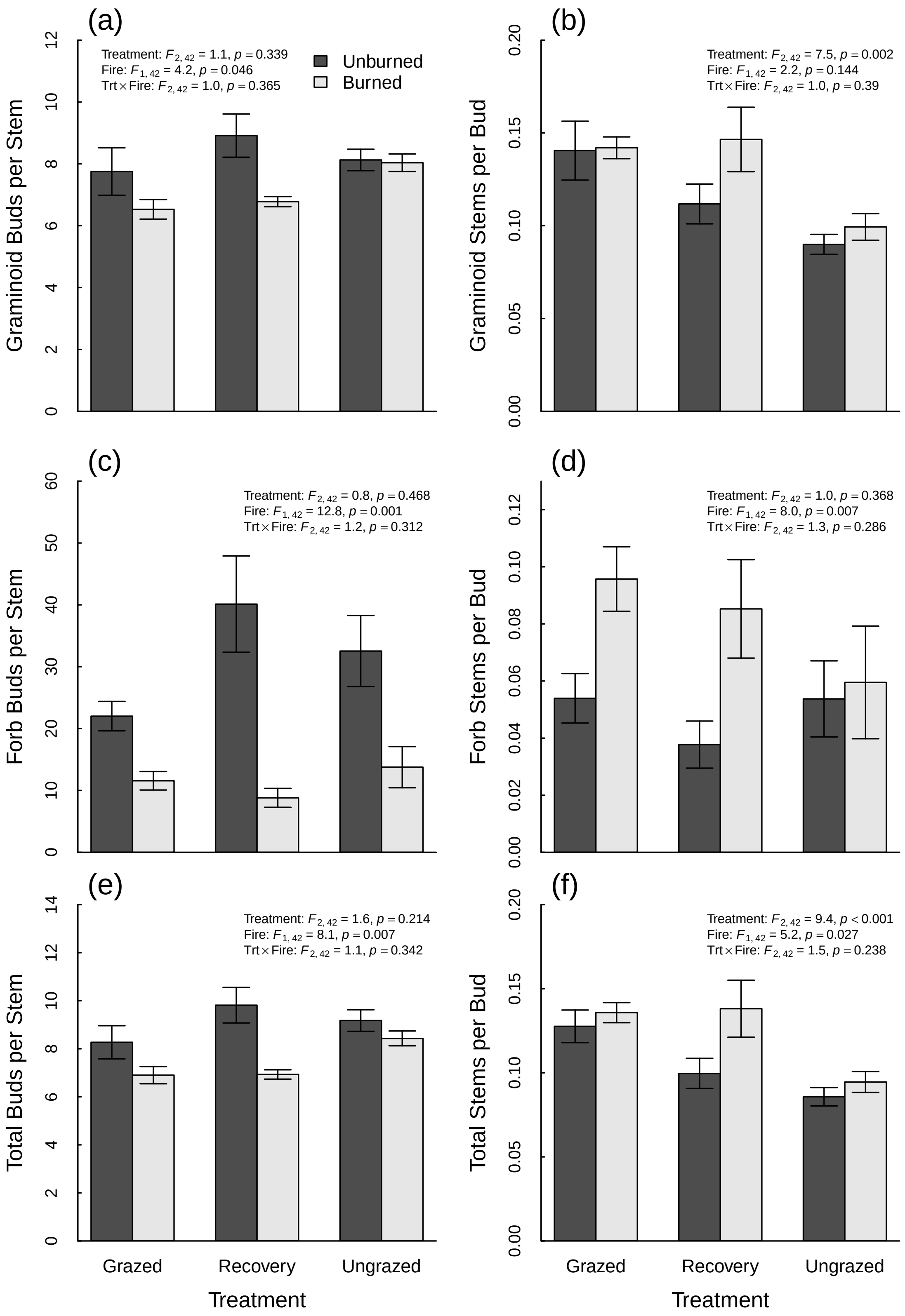

Figure 3.6: Effect of 2011 fire on the bud-to-stem transition (a, c, e) and stem-to-bud transition (b, d, f) for graminoids, forbs, and total. Bars are means ± 1 SE.

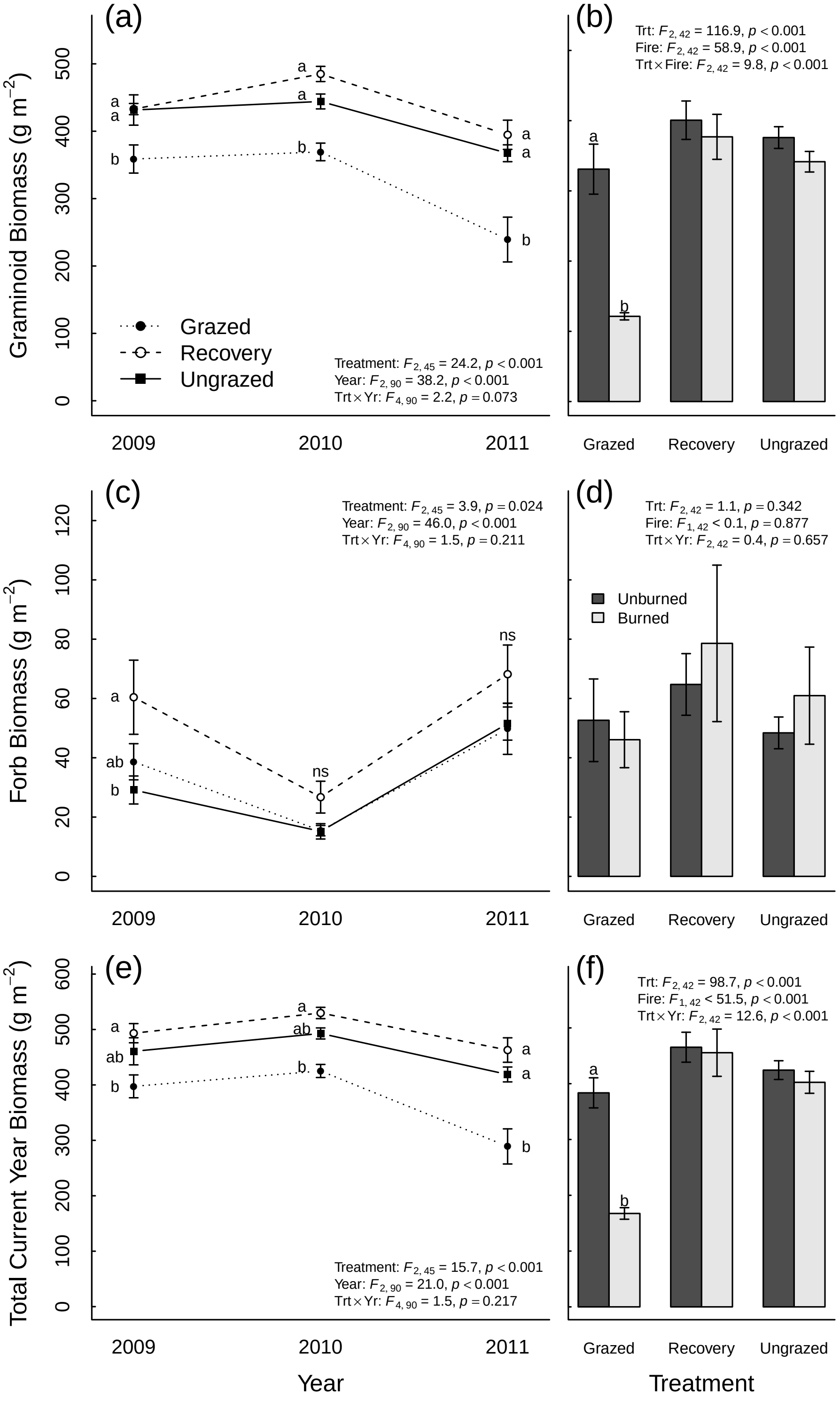

Figure 3.7: Graminoid (a, b), forb, (c, d) and total (e, f) aboveground biomass production for 2009 to 2011. Biomass in grazed areas is residual end of season biomass, and does not account for herbivory by cattle. The second column (b, d, f) shows the effect of fire on biomass production in 2011. Points and bars are means ± 1 SE.

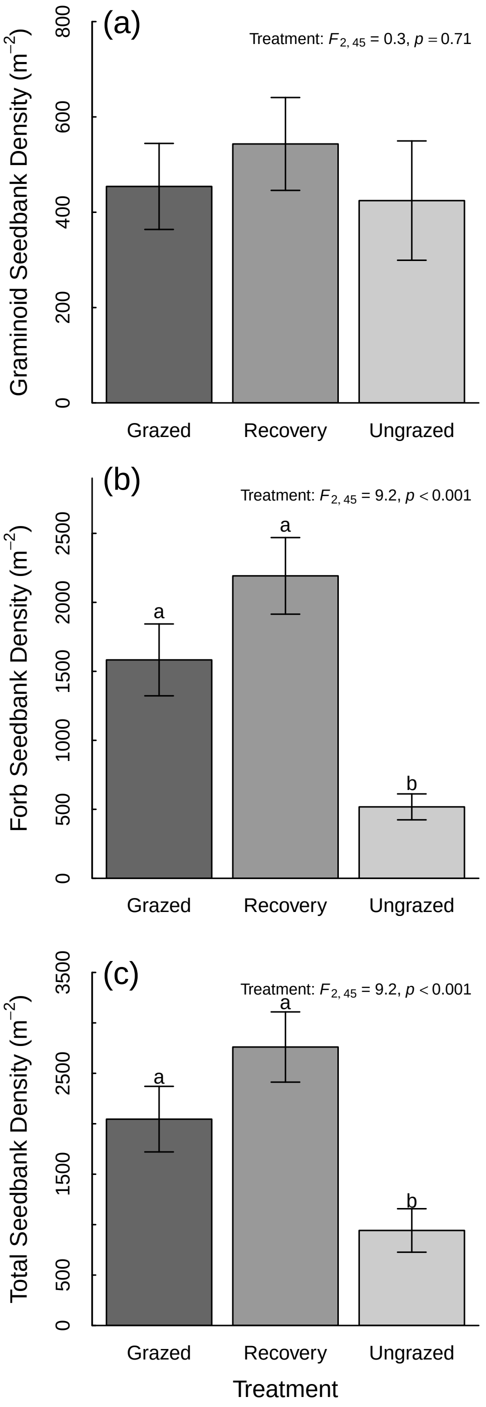

Figure 3.8: Graminoid (a), forb (b), and total seed bank density by treatment sampled in April 2012 after the 2011 growing season. Bars are means ± 1 SE.

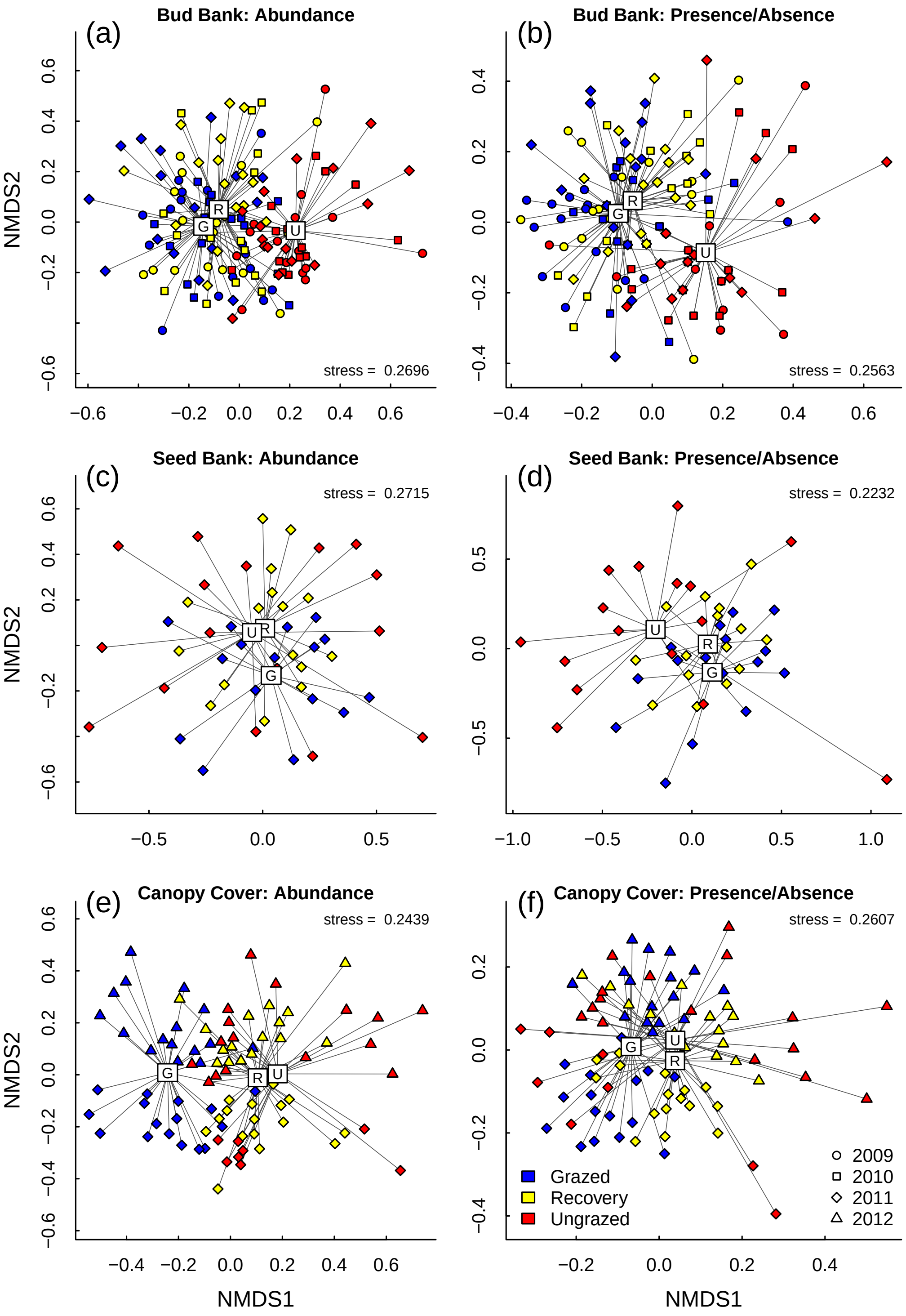

Figure 3.9: Non-metric multi-dimensional scaling (NMDS) ordinations for the bud bank from 2009 to 2011 (a, b), the seed bank after the 2011-2012 dormant season (c, d), and canopy cover for 2011 and 2012 (e, f) portions of the plant community. Ordinations in the left column were produced using the quantitative (abundance) form of the Bray-Curtis index, while the right column was produced using the binary (presence-absence) form of the Bray-Curtis index. Centroids of each treatment are labeled, with G = Grazed, R = Recovery, and U = Ungrazed. Stress is type 1, as in Kruskal (1964).

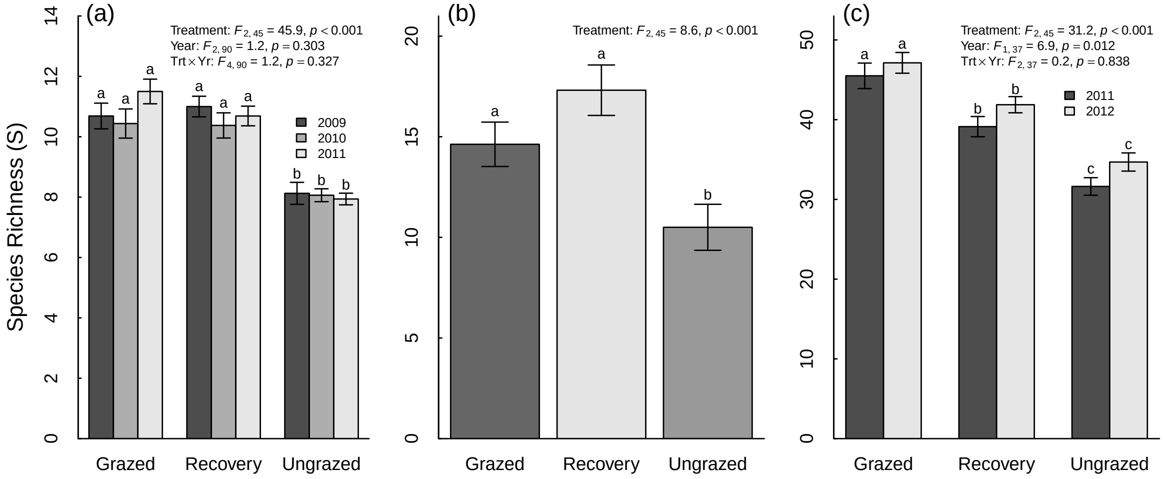

Figure 3.10: Species richness (S) for (a) bud bank, with richness per 0.01 m^2 soil core; (b) seed bank, with richness per 0.029 m^2; and (c) canopy cover, per 10 m^2 circular quadrat. The bud bank was sampled from 2009 to 2011, the seed bank was sampled in spring 2012, and aboveground canopy cover was sampled in 2011 and 2012. Bars are means ± 1 SE.

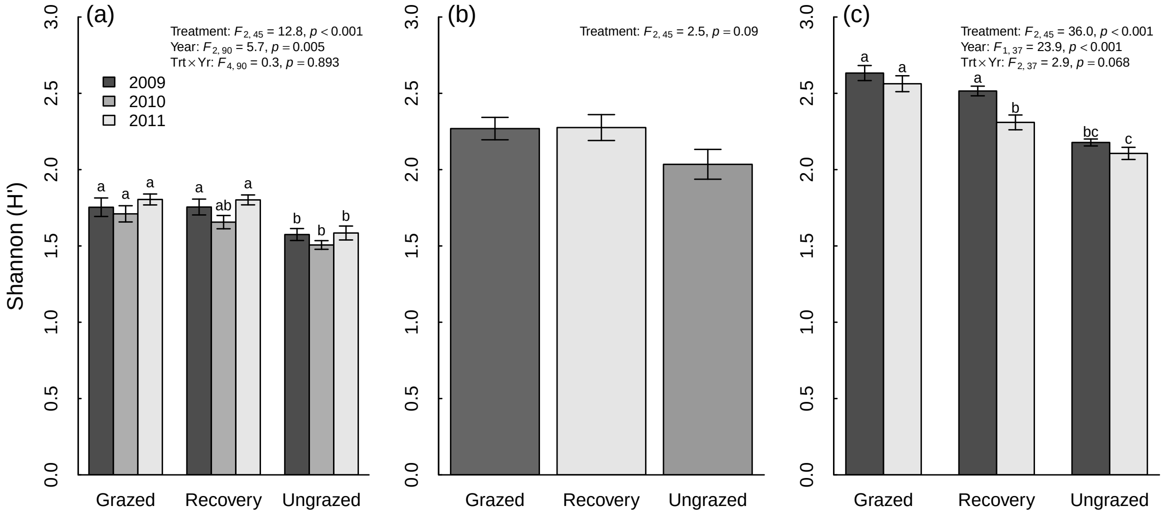

Figure 3.11: Shannon diversity (H') for (a) bud bank, (b) seed bank, and (c) canopy cover. Letters indicate significant differences among grazing treatments within years. The bud bank was sampled from 2009 to 2011, the seed bank was sampled in spring 2012, and aboveground canopy cover was sampled in 2011 and 2012. Bars are means ± 1 SE.

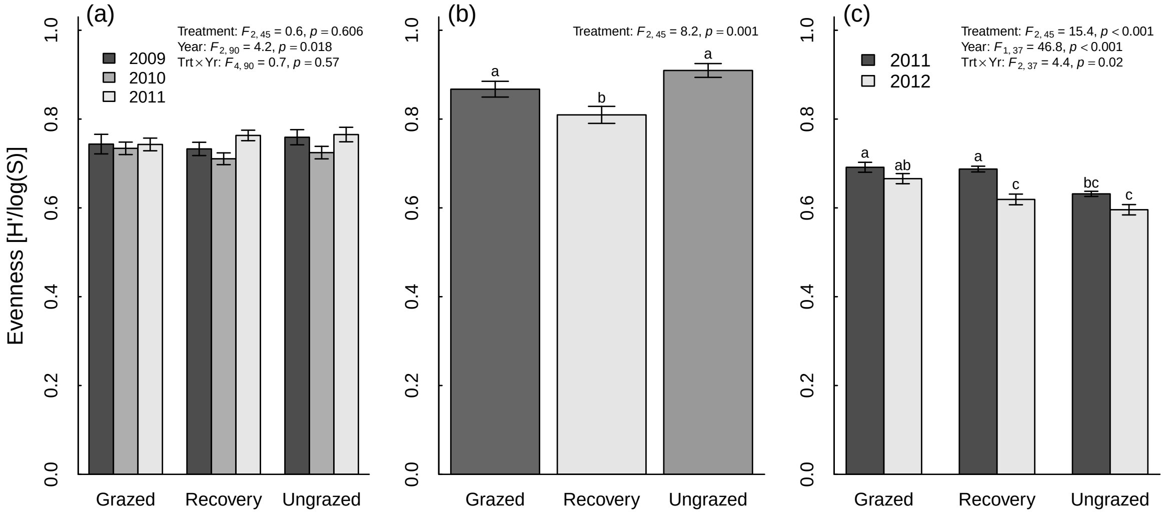

Figure 3.12: Evenness ($H'/\ln(S)$) for (a) bud bank, (b) seed bank, and (c) canopy cover. The bud bank was sampled to species from 2009 to 2011, the seed bank was sampled in spring 2012, and canopy cover was sampled in 2011 and 2012. Bars are means ± 1 SE.

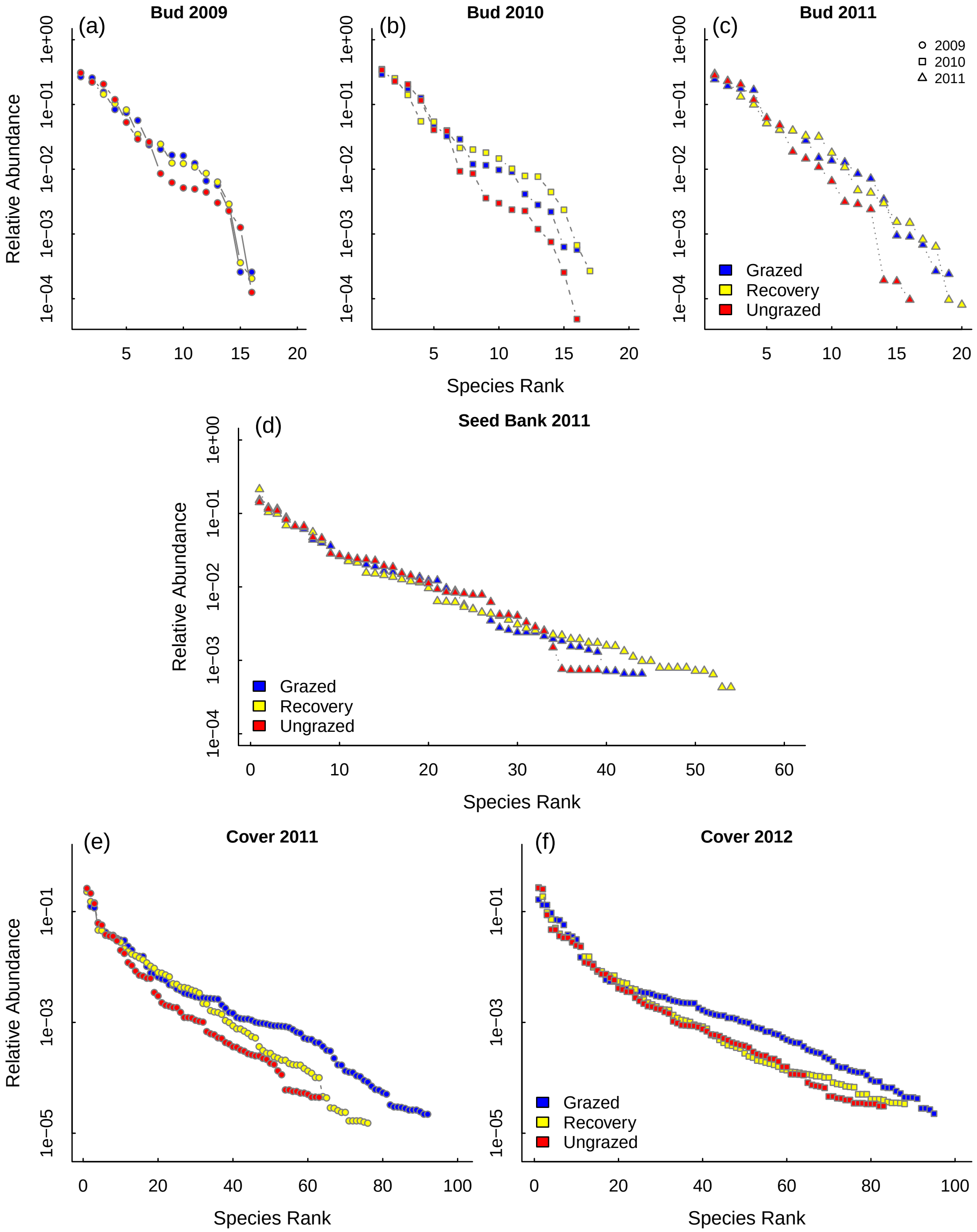

Figure 3.13: Rank-abundance diagrams for the bud bank (a, b, c), seed bank (d), and canopy cover (e, f). The y-axis is plotted on a log scale.

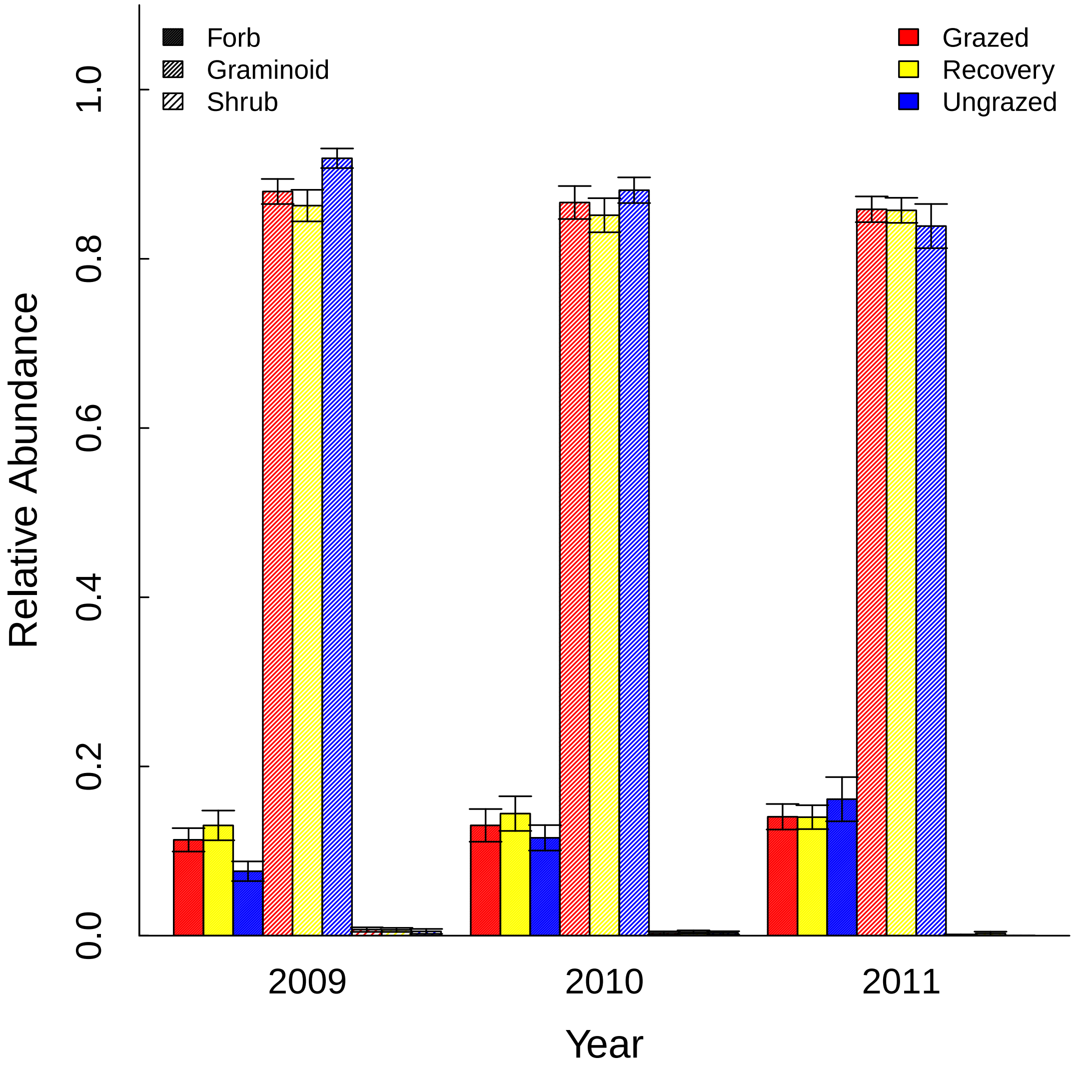

Figure 3.14: Relative abundance of growth forms in the bud bank, 2009 to 2011. Summary statistics are given in the text. Colors indicate treatments, while the density of hatching indicates growth form. Bars are means ± 1 SE.

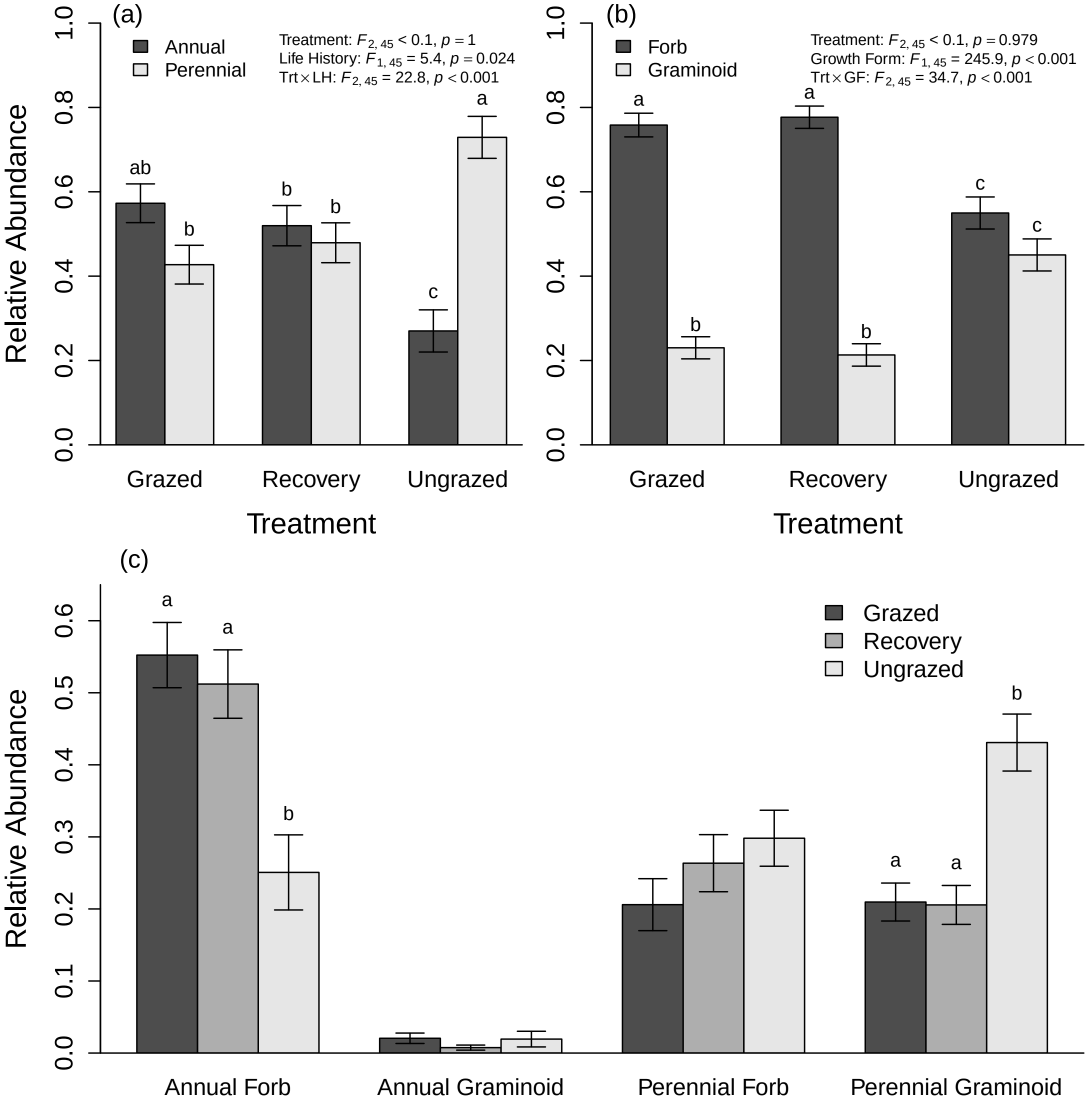

Figure 3.15: Relative abundance in the seed bank of (a) annual and perennial life history strategies, (b) graminoid and forb growth forms, and (c) the four life history × growth form groups. In (a) and (b), different letters indicate significant differences among all groups, while in (c) different letters indicate significant differences within life history × growth form groups. *LH* indicates "life history", and *GF* indicates "growth form." Bars are means ± 1 SE.

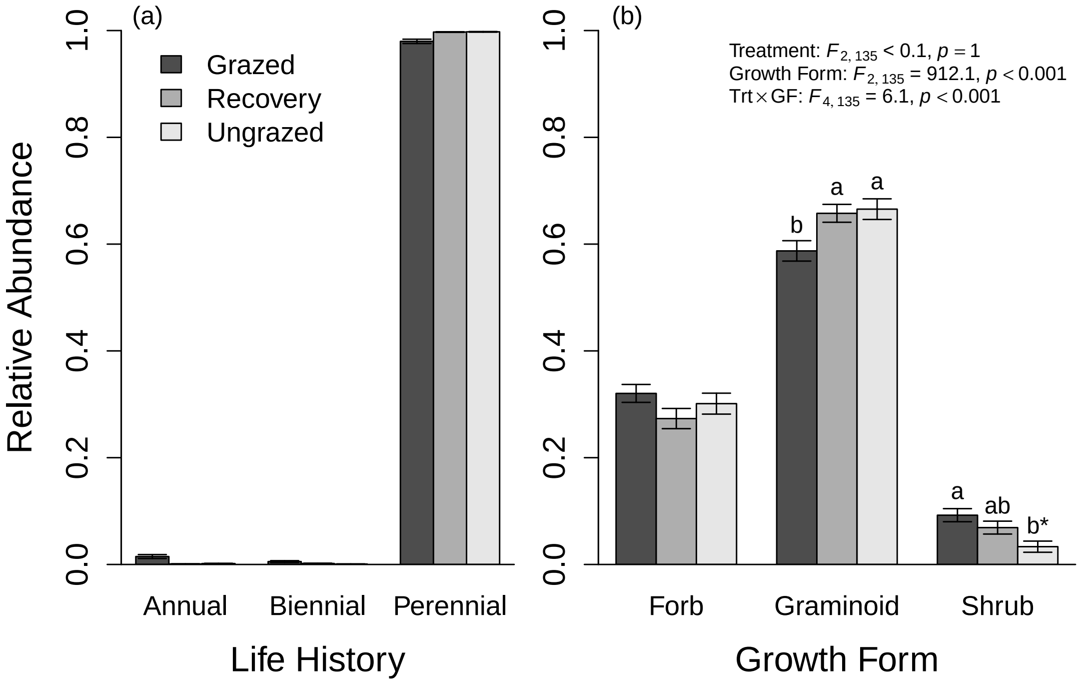

Figure 3.16: Aboveground relative abundance of (a) life history strategies and (b) growth forms, using canopy cover to estimate abundance. In (b), different letters indicate significant differences ($p < 0.05$) within growth forms, correcting for six multiple comparisons with the Bonferonni method; "b*" indicates that the difference is significant at $p < 0.1$. Bars are means ± 1 SE.

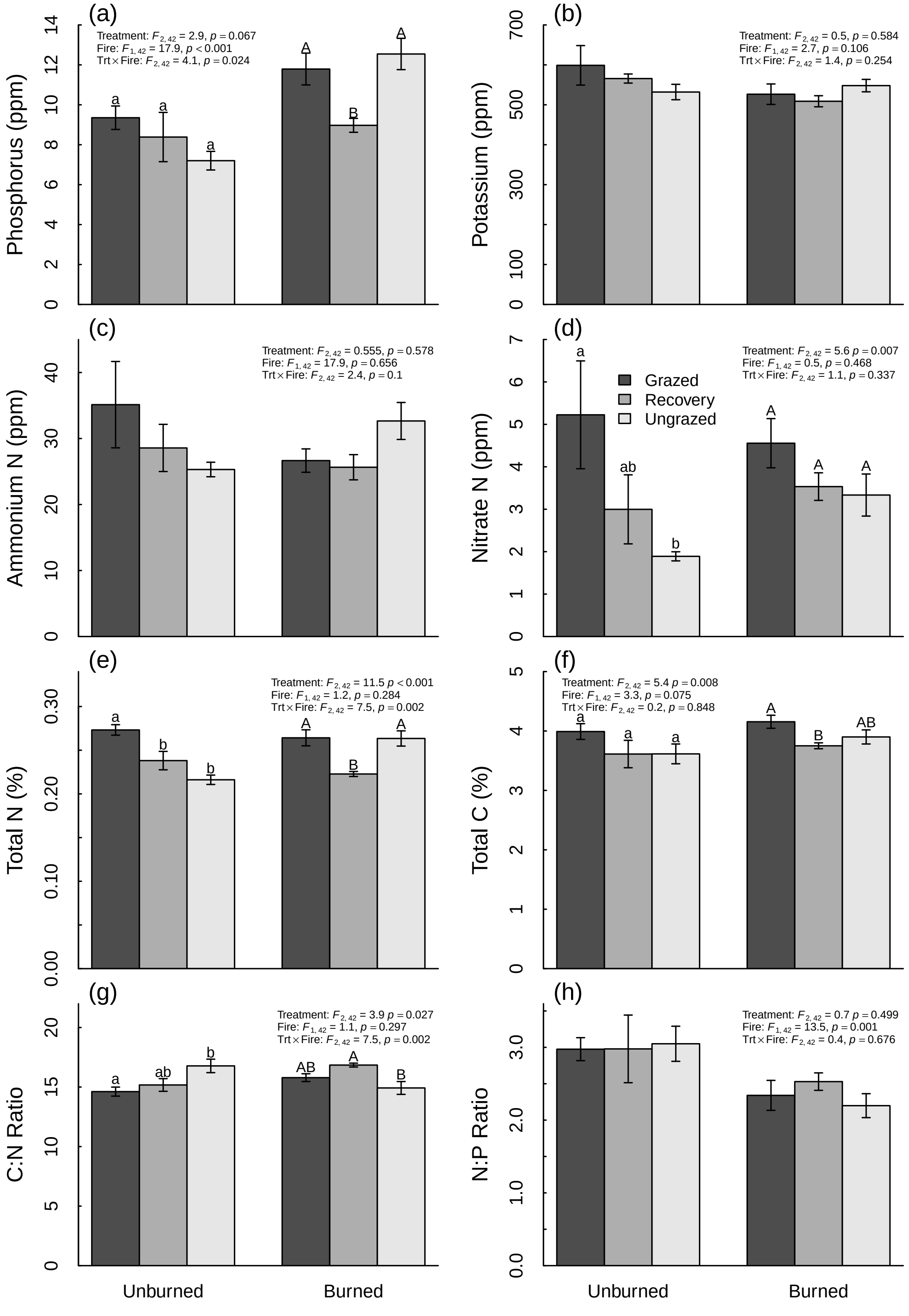

Figure 3.17: Legacy effects of grazing on soil nutrients. Because experimental units were not all burned the year prior to sampling, soil nutrient levels are shown separately for burned and unburned areas. Dark gray bars indicate grazed areas, medium gray bars indicate recovery areas, and light gray bars indicate ungrazed areas. Bars are means ± 1 SE.

Table 3.1: Counts of emerged seedlings by species and treatment.

Summary	Grazed	Recovery	Ungrazed
Total	964	1301	444
Total identified	786	1116	395
Total unidentified	178	189	50
Total forb	746	1033	244
Total graminoid	214	256	200
Total woody	4	12	0
Unidentified forb	153	165	38
Unidentified graminoid	25	24	12
Taxon	Grazed	Recovery	Ungrazed
Acalypha virginica	0	0	2
Agrostis hyemalis	12	30	16
Amaranthus rudis	1	0	1
Ambrosia psilostachya	10	14	14
Amorpha canescens	2	2	0
Androsace occidentalis	10	3	3
Artemisia ludoviciana	1	5	3
Astragalus sp.	1	0	0
Carex gravida	2	6	14
Chamaesyce maculata	3	6	3
Chamaesyce nutans	13	27	4
Chamaesyce prostrata	0	0	1
Chloris verticillata	2	5	0
Conyza canadensis	0	1	3
Corydalis micrantha	1	2	0
Croton capitatus	0	0	1
Cyperus lupulinus	14	14	8
Cyperus squarrosus	1	1	0
Dalea purpurea	7	5	3
Dichanthelium oligosanthes	46	63	46
Draba cuneifolia	1	0	0
Draba reptans	39	20	0
Eragrostis spectabilis	22	10	8
Erigeron strigosus	115	190	11
Euphorbia sp.	1	1	1
Juncus interior	11	30	58
Koeleria macrantha	28	16	1
Lepidium densiflorum	1	1	1
Lepidium virginianum	35	38	2
Linum sulcatum	9	12	6
Mollugo verticillata	1	1	0

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Table 3.1 – continued from previous page

Taxon	Grazed	Recovery	Ungrazed
Monolepis nuttalliana	0	1	0
Oxalis stricta	85	122	39
Panicum capillare	0	1	0
Plantago rhodosperma	56	97	4
Poa pratensis	2	6	6
Populus deltoides	0	3	0
Pseudognaphalium obtusifolium	1	2	2
Ratibida columnifera	1	3	0
Salix amygdaloides	2	3	0
Salix exigua	0	3	0
Schizachyrium scoparium	0	4	0
Silene antirrhina	80	58	18
Solanum rostratum	0	0	1
Solidago canadensis	13	126	36
Sphenopholis obtusata	12	16	1
Sporobolus compositus	11	22	24
Sporobolus cryptandrus	2	0	1
Symphyotrichum ericoides	10	45	38
Teucrium canadense	0	5	0
Triodanis leptocarpa	0	3	0
Triodanis perfoliata	94	70	7
Typha latifolia	1	0	0
Ulmus sp.	0	1	0
Verbascum blattaria	0	1	1
Verbena stricta	2	7	1
Vernonia baldwinii	1	1	0
Viola bicolor	0	1	0
Vulpia octoflora	24	8	5

Chapter 4

Drought has greater effects than grazing on tallgrass prairie vegetation dynamics

Abstract

While the effects of drought and grazing are often studied separately, these disturbances interactively influence population, community, and ecosystem processes in grasslands. I investigated the combined effects of drought and grazing on above to and belowground vegetation dynamics of tallgrass prairie in northeast Kansas. Because herbaceous plants re to sprout from belowground buds after disturbance, I was particularly interested in the stability of the belowground bud bank when perturbed separately and interactively by drought and grazing. I crossed two levels of drought (ambient versus 80% precipitation interception) and two levels of grazing (ungrazed versus clipping graminoids three times during the growing season) in a factorial treatment structure. The experimental drought was applied on top of a severe natural drought in 2012, presenting the unique opportunity to test vegetation responses to severe drought conditions which may become more common in the future. I found that aboveground plant community composition shifted in response to drought, while belowground bud bank composition shifted in response to grazing. Species richness declined both above to and belowground in drought plots relative to ambient plots. These changes in community composition were driven by differential responses of grasses, sedges, and forbs to drought and grazing treatments. Graminoid aboveground net primary productivity (ANPP) was 5 to 15% greater when grazed, but across grazing treatments declined 30 to 50% under drought conditions. Forb ANPP was greatest under grazing and ambient precipitation, with no difference

among other treatment combinations. Drought reduced seedling survival, with 35 to 40% survival under ambient conditions but only 20 to 25% survival under drought, regardless of grazing treatment. C_4 grass flowering stem density was reduced to near zero under drought, with little additional effect of grazing. Grass bud and stem densities remained constant (approximately 80 to 90 buds and 10 to 15 stems per 0.01 m^2) under all drought and grazing treatments. Finally, I demonstrated that live rhizome biomass is highly correlated bud bank density, and that live rhizome biomass can be used to accurately predict bud bank density across drought and grazing conditions. I conclude that changes in vegetation were driven primarily by drought, with few additional effects of grazing either separately or interactively.

4.1 Introduction

Grasslands and grass-dominated ecosystems cover nearly one-third of the earth's surface, providing important habitat for a diverse flora and fauna, delivering important ecosystem services, and supporting a large human population (Curtin and Western 2008). However, much of the native grassland has been lost to agriculture (Samson and Knopf 1994), and remnant grasslands are being degraded by intensive grazing by domesticated cattle (McNaughton 1993). Humans have replaced many of the native grazers with domesticated grazers such as cattle, sheep, and goats, often altering not only the identity of the grazers, but also their density, spatial distribution, and timing (McNaughton 1993, Frank and Groffman 1998). While grassland vegetation tolerates grazing because of the long, shared evolutionary history with grazers (Nilsson et al. 1996, Lehtila 2000, Anderson 2006), altered grazing regimes may exacerbate damage from other disturbances. Extreme drought is expected to increase in grasslands worldwide (Min et al. 2011) and recent studies predict changes in temperature and precipitation amount and timing globally and in the Great Plains of North America (Patricola and Cook 2013). Therefore, mechanistic explanations of vegetation response to extreme drought and grazing will allow land managers to adaptively manage grazers and promote long-term sustainability of grassland systems.

Both drought and grazing cause shifts in grassland plant community composition. Large ungulate grazers increase spatial heterogeneity and species diversity through both their feeding (e.g. selective consumption of certain species) and non-feeding (e.g. hoof action, wallowing) behaviors

(Hartnett et al. 1996, Biondini et al. 1998). Long-term grazing has long been known to reduce productivity and root growth in various grassland systems: Biswell and Weaver (1933) review the work of 22 studies on this topic, and numerous other studies have been done in the following 80 years confirming these results and developing detailed explanations. By preferentially feeding on grasses, grazers release forbs from competitive dominance by grasses and increase opportunities for seedlings to establish. In tallgrass prairie grazing also reduces density of the bud bank (Chapter 3), the source of most aboveground stems in both undisturbed and disturbed areas of this grassland (Benson and Hartnett 2006, Rogers and Hartnett 2001) and an important component in plant response to herbivory (Nilsson et al. 1996).

Drought also differentially affects plant species. Several key traits are associated with relative drought tolerance, including hydraulic architecture, morphology, and rooting depth, affecting plant growth and survival during periods of high water stress (Tucker et al. 2011, Weaver and Albertson 1936). Many species tolerate short-term drought. In Chapter 2 I found that while a 2-year experimental drought reduced productivity and shifted plant community composition, it did not reduce bud bank density. As length and severity of drought increases, even deep-rooted species suffer mortality and are replaced by opportunistic species that colonize canopy openings (Weaver and Hansen 1939, Weaver and Mueller 1942). Thus drought causes changes in community composition both during and after the event through differences in growth, survival, and establishment of plant species.

Maintaining grazers on a grassland during extreme drought events may have little additional effect on vegetation dynamics beyond the effects of drought (Biondini et al. 1998). When plants senesce aboveground tissue in response to water stress (Volaire et al. 2009), they translocate nonstructural carbon from aboveground tissue to storage organs, facilitating future regrowth from belowground buds (Busso et al. 1989). If plants senesce aboveground tissue to prevent complete desiccation and mortality during drought, grazing that removes this dead tissue will have little additional effect on the belowground bud bank. Furthermore, if plants form new belowground buds early in the growing season, before moisture stress becomes severe and before grazers are placed on the pasture, removal of aboveground biomass by grazers may have little effect on bud production or total plant community composition.

Alternatively, grazing during severe drought may increase degradation of grassland relative to ungrazed areas (Zwicke et al. 2013, Illius and O'Connor 1999), perhaps by decreasing belowground carbon storage (McSherry and Ritchie 2013) and further reducing production of belowground buds (Chapter 3). Plants that are already stressed by drought may suffer greater mortality when grazed, reducing overall density of stems (Koerner et al. 2013). If plants have not fully senesced their aboveground tissue, loss of this tissue to grazing may represent a significant loss of fixed carbon and nutrients. As a result, bud bank density may also decline to a greater extent in grazed areas that are droughted than in non-droughted areas if grazing drought-stressed plants induces additional damage to plants.

In this research my primary question was "How does the density, demography, and composition of the bud bank change in response to grazing and drought?" While short-term responses of aboveground community composition and productivity document immediate responses to these disturbances, changes in the bud bank may better predict longer-term responses and future change in community composition. I addressed this question by testing the hypotheses presented above to document the patterns and processes of vegetation responses to drought and grazing. I implemented a factorial treatment design which crossed two levels of precipitation (ambient, drought) with two levels of grazing (ungrazed, grazed) and documented above- and belowground plant responses to these treatments.

4.2 Methods

4.2.1 Site Description

Konza Prairie Biological Station (KPBS) is a 3487 ha tallgrass prairie preserve in the Flint Hills of northeast Kansas, USA (39°05′ N, 96°35′ W). KPBS experiences a continental climate, with warm, wet summers and cool, dry winters. Mean monthly air temperatures range from -2.7 °C, in January to 26.6 °C in July. Total annual precipitation averages 880 mm [1983-2012, NOAA National Climate Data Center (2013)], with 79% falling during the growing season (April to October). However, precipitation patterns in this region are characterized by high variability among years, ranging from 513 to 1435 mm annually in the period 1983-2012. Annual precipitation was below average for 2012 (570 mm), with 74% falling during the growing season (Konza HQ1MET weather station). A matrix of C_4 grasses dominates the vegetation, including big bluestem (*Andropogon gerardii* Vit.), Indian grass (*Sorghastrum nutans* [L.] Nash), and little bluestem (*Schizachyrium scoparium* Michx.), with a wide variety of subdominant C_4 grasses, C_3 grasses, and forbs interspersed (Towne 2002). KPBS has steep topography, with shallow upland cherty silt loam soil and deeper lowland silt clay loam soil.

Native (never plowed) upland tallgrass prairie covers the unit of KPBS in which this study was conducted. The study area was ungrazed during the 1970s and 1980s, and was lightly grazed by cattle (*Bos taurus*) during the growing season in 1992 and 1993 (6.1 ha per cow/calf). From 1994 to 2007, these areas were grazed at 1.7 ha/animal unit by yearling Angus by Hereford steers, but have not been grazed since. This unit was burned on average every two years until 1994. From 1994 until 2009, the unit was burned annually in March or April. The unit was not burned in 2010 and 2011, but was burned the year of the study, 2012.

4.2.2 Precipitation and Grazing Treatments

I randomly assigned precipitation treatments (80% precipitation interception versus ambient precipitation) to forty-eight 2 by 2 m plots on relatively level upland tallgrass prairie. This experimental manipulation of precipitation, combined with the severe natural drought, presented the unique opportunity to test vegetation responses under extreme drought conditions. At least 3 m separated plots from each other. I used passive rainfall interception structures to exclude 80% of the incident precipitation on drought plots from mid-March to October of 2012 using a modification of the shelters tested by Yahdjian and Sala (2002). Shelters measured 2.4 by 2.4 m, with 2.4 m by 0.2 m clear Lexan shingles (SABIC Innovative Plastics, Pittsfield, Massachusetts, USA) sloping to the south at 15° to account for prevailing southerly winds in the summer. Shingles were bent lengthwise at 120° and spaced to cover 80% of the total area of the shelter (Fig. 4.1). Using an Analytical Spectral Devices (ASD) FieldSpec Pro portable spectrometer (Boulder, Colorado, USA), I determined that at least 90% of visible light was transmitted by the shingles. All water collected by the shingles was drained at least 2 m away from the shelter on the downhill side and away from other plots.

I randomly assigned grazing treatments (grazed versus not grazed) to plots within precipitation treatments. I simulated grazing by selectively clipping graminoids (grasses, sedges, and rushes) to 5 cm above the soil surface using scissors. I originally planned to clip every three weeks, starting in May, but I was only able to clip on 17 May, 12 June, and 3 July because grasses grew very little for the remainder of the growing season due to extreme hot, dry weather. Clipping removed 42.7±1.6% of aboveground biomass in ambient plots, and 54.0±2.6% of biomass in drought plots (mean ± SE). Due to an error in assigning treatments to plots, ambient/grazed and drought/ungrazed treatment combinations were assigned to thirteen plots each, while ambient/ungrazed and drought/grazed treatments were assigned to eleven plots each. Clipped biomass from the central 1 by 1 m area of grazed plots was collected, dried at 60 °C for at least 48 h and weighed to the nearest 0.01 g. Total end-of-season aboveground biomass of both grazed and ungrazed plots was harvested on 17 September by clipping at the soil surface. I used end-of-season biomass from unclipped plots to estimate aboveground net primary productivity (ANPP), while for clipped plots I pooled end-of-season biomass with all biomass collected from earlier clipping dates.

4.2.3 Plant Community Sampling

All plant community sampling occurred in the central 1 by 1 m area of each plot to minimize edge effects. End-of-season stem density was estimated in September 2012 by counting and identifying stems to species in four 10 by 10 cm quadrats randomly located within plots. These quadrats were then marked with pin flags in opposite corners until soil cores were harvested. To estimate bud bank density and species composition, I harvested four 10×10 cm soil cores directly below the stem-count quadrats to a depth of 10 cm. I harvested soil cores harvested between 27 to 29 November 2012, immediately placed them in plastic bags, and stored at 4 °C pending processing. Processing involved rinsing soil from the belowground samples (no more than 3 weeks prior to examination) and examining belowground plant organs using a dissecting microscope, trimming roots to allow thorough examination of the belowground structures. Belowground buds were counted and assigned to species using bud morphology, phyllotaxy, morphology of the attached root system, and morphology of any remaining aboveground parts, as in Carter et al. (2012). After identifying buds to species, I estimated living belowground rhizome biomass for each species by drying live rhizomes at 60 °C and weighing to the nearest 0.01 g.

I sampled aboveground plant community composition in May and August of 2012 in two square 0.25 m^2 quadrats in opposite corners of the central 1 by 1 m area of each plot. I counted stems to species and estimated cover of each species using modified Daubenmire cover classes. I counted all seedlings present in these quadrats in May, and marked up to 30 seedlings of each species per plot with galvanized nails. I then monitored seedling survival monthly from May until mid-September to estimate seedling survival over the growing season.

4.2.4 Statistical Analyses

I performed all data analyses with R 3.0 (R Core Team 2013). Plots initially appeared homogeneous when installed, but detailed vegetation characterization as the plant canopy developed suggested that plots should be blocked by location for analysis. I performed non-metric multidimensional scaling ordinations (NMDS) to confirm blocking, using the `metaMDS` routine in the *vegan* package (Oksanen et al. 2013). Species scores were square root transformed and Wisconsin double standardized prior to analysis (Bray and Curtis 1957). Pairwise dissimilarity between plant communities (plots) was computed using the Bray-Curtis index, using species aboveground stem and belowground bud abundances. NMDS ordinations confirmed that plots fell into two groups, both above- and belowground (Fig. 4.2), consistent with plot location within the study area. The first block included nine ambient, grazed plots; six ambient, ungrazed plots; seven drought, grazed plots, and 10 drought, ungrazed plots. The second block included five ambient, grazed plots; four ambient, ungrazed plots; four drought, grazed plots, and three drought, ungrazed plots. I included these two location blocks in a mixed model for bud density, stem density, buds per stem, ANPP, and seedling survival, but I found that for responses that did not include species-specific information, mixed models accounting for blocks did not perform better than models without blocking. Blocking was therefore only retained for analysis of plant community composition.

I evaluated shifts in below- and aboveground community composition and diversity in response to drought and grazing treatments using mean bud bank density from the four 0.01 m^2 soil cores per plot and mean end-of-season aboveground stem density from the 0.01 m^2 area directly above each soil core. I used partial distance-based redundancy analysis (partial db-RDA), a constrained ordination technique on the community dissimilarity matrix that is able to remove the effect of a random variable before performing the RDA (Legendre and Gallagher 2001). I used the `capscale` function in the *vegan* package (Oksanen et al. 2013). Dissimilarity between sites was calculated using the quantitative (abundance) form of the Bray-Curtis index. I modeled the community response as a function of the drought and grazing main effects and their interaction, blocking by location as described above. I assessed the effect of drought and grazing on community composition using permutational test of the terms in the db-RDA model, with 10,000 permutations. To test the effect of treatments on below- and aboveground diversity, I estimated species richness (S, total species per plot), Shannon diversity (H'), and evenness (H'/log[S]).

Responses of ANPP, bud density, stem density, percent difference in stem density (between May and August), demographic rates, C_4 grass flowering stem density and belowground rhizome biomass were analyzed using analysis of variance. I tested the assumption of normality using visual examination of histograms and normal-quantile plots, and with the Shapiro-Wilk test. When not normal, I performed generalized linear modeling using the gamma distribution (inverse link) for continuous variables and the negative binomial distribution (log link) for counts, using the *glm* function in R. I used Type III sums of squares to account for slightly unbalanced sample sizes.

I used nonparametric maximum likelihood estimators to estimate the distribution function for survivorship of seedlings in response to drought and grazing treatment combinations. This method can be applied to interval-censored data, which occurred in this study because the interval containing the date of seedling death was known, but not the exact date. These seedling survival data were right-censored for seedlings that survived until the final sampling date ($n = 580$) and interval-censored if a seedling died during a preceding interval ($n = 1297$). After estimating the distribution function, I used parametric regression (*survreg* function in the *survival* package in R) to determine the effects of drought and grazing treatments on seedling survival, which is an accelerated failure time (AFT) model in R. Seedlings within plots were modeled as non-independent units. The

Weibull distribution was chosen for the parametric model because it maximized the log likelihood. Survival analyses were done using the *interval* and *survival* packages for R (Fay and Shaw 2010, Therneau 2013).

4.3 Results

I found that both below- and aboveground community composition and diversity shifted in response to drought and grazing treatments. Differential responses of species to drought and grazing drove change in composition and diversity. Grass stem and bud bank density did not change in response to drought or grazing treatments. In contrast, sedge and forb bud and stem densities responded to both drought and grazing. I demonstrated that the response of live rhizome biomass closely mirrors the bud bank response, and that live rhizome biomass can be used to accurately predict bud bank densities. In many cases drought and grazing together did not drive greater changes than applying these treatments separately. Overall, drought had a greater effect on vegetation dynamics than grazing (Table 4.3).

4.3.1 Plant Community Composition and Diversity

Analysis of above- and belowground plant abundance data suggests that community composition shifted within one growing season in response to drought and grazing treatments. The aboveground plant community composition shift in response to drought was marginally significant ($F_{1,43} = 1.64$, $p = 0.071$), but there was no effect of grazing or the interaction of drought and grazing ($F_{1,43} = 1.32$, $p = 0.184$; $F_{1,43} = 1.46$, $p = 0.126$; respectively. Fig. 4.3a). The effect of grazing on belowground community composition was also marginally significant ($F_{1,43} = 1.59$, $p = 0.086$), but there was no effect of drought or the interaction of drought and grazing ($F_{1,43} = 1.24$, $p = 0.236$; $F_{1,43} = 0.62$, $p = 0.832$; respectively. Fig. 4.3b).

Drought appeared to be the primary treatment factor affecting above- and belowground diversity. Species richness declined 15 to 20% both above- and belowground across grazing treatments in response to drought (Fig. 4.4a, b). Shannon diversity (H') declined slightly belowground in response to drought, with no effect of grazing. Aboveground drought and grazing interactively affected diversity: grazed plots did not differ in H' across precipitation treatments, but ungrazed, ambient plots had greater diversity than ungrazed, droughted plots (Fig. 4.4c, d). There were no changes in evenness (J) belowground in the bud bank in response to drought and grazing treatments. Aboveground, changes in evenness reflected changes in diversity (H'), with no difference between grazed plots, but greater evenness in ambient, ungrazed plots relative to droughted, ungrazed plots (Fig. 4.4e, f).

4.3.2 Aboveground Responses

Aboveground Net Primary Productivity

Drought and grazing interactively affected total ANPP. Total ANPP was reduced 25 to 35% by drought relative to ambient plots. However, under ambient conditions ANPP was 40% greater in grazed plots relative to ungrazed plots, while grazing had no effect on productivity in drought plots (Fig. 4.5a). Graminoid ANPP was reduced 30 to 40% in drought plots relative to ambient plots, with a slight increase in ANPP in both drought and ambient plots due to grazing (Fig. 4.5b). Forb ANPP in ambient, grazed plots was two times greater than all over plots (approximately 100 versus 50 g m^{-2}), with no difference in forb ANPP across all other treatment combinations (Fig. 4.5c).

Seedling Survival

Drought reduced the probability of seedling survival, with no effect of grazing despite small differences between grazed and ungrazed droughted plots early in the growing season, and between grazed and ungrazed ambient plots later in the growing season (Table 4.1, Fig. 4.6). Seedling death was particularly rapid during the month of July for drought plots, with a 30 to 40% drop in survivorship during this period compared to a 15 to 20% drop in ambient plots (Fig. 4.6a). The probability of surviving to the end of the growing season reflects season-long survivorship patterns, with the lowest probability of survival in seedlings in drought plots (20-25%) relative to seedlings in ambient plots (35 to 45%, Fig. 4.6b).

Stem Density, Senescence, and Flowering

Drought reduced total end to of to season stem density by 10 to 20%, but grazing did not affect total stem density (Fig. 4.7a). Analyzing grass, sedge, and forb stem densities separately revealed that each group responds differently to drought and grazing. Neither drought nor grazing reduced grass stem densities (Fig. 4.7c). Sedge stem density was reduced approximately 20% by drought, with no effect of grazing (Fig. 4.7e). Although neither drought nor grazing affected forb stem density, grazed areas consistently had greater median stem density than ungrazed areas(Fig. 4.7g).

Across all treatments, plants senesced approximately 10 to 30% of aboveground stems from May to August (Fig. 4.8). Despite a significant drought by grazing interaction, the primary difference was between drought treatments. Under ambient precipitation, approximately 10 to 20% of stems senesced, while under the drought, 25 to 35% of their stems died over the growing season. Closer examination of grasses, sedges, and forbs revealed these groups responded differently to drought and grazing treatments (Fig. 4.8b). Forbs senesced 20 to 30% of their stems across all treatments. Survival of grass stems was reduced approximately 10% in response to drought, with little difference between grazing treatments. Under ambient conditions grazed sedges recruited new stems over the growing season, but ungrazed sedges senesced approximately 15% of stems. Under drought 30% of sedge stems senesced, regardless of grazing treatment.

The response of warm to season (C_4) grass flowering stem density depended on both drought and grazing treatments, but was primarily reduced by drought (Fig. 4.9). There was no difference in C_4 grass flowering stem density in ambient plots. When only droughted, some C_4 grasses still flowered, but when both droughted and grazed, C_4 grass flowering was eliminated from nearly all plots. *Sporobolus compositus* (Poir.) Merr. accounted for 63% of all C_4 grass flowering stems and *Bouteloua curtipendula* (Michx.) Torr. accounted for 15% of flowering stems, with various other grass species contributing less than 5% of total flowering stems.

4.3.3 Belowground Responses

Bud Density and Demography

Similar to total stem density, drought reduced total bud density by approximately 20% relative to ambient, with no effect of grazing (Fig. 4.7b). However, grass, sedge, and forb bud bank densities did not respond similarly to drought and grazing treatments. Like stem density, neither drought nor grazing affected grass bud densities (Fig. 4.7d). Drought and grazing had an interactive effect on sedge bud density: drought reduced sedge bud density to nearly half of ambient density, with the greatest decline in bud density in plots that were droughted but not grazed (Fig. 4.7f). Forb bud density declined when droughted, but did not respond to grazing (Fig. 4.7h).

Buds per stem is a measure of bud production standardized by the density of aboveground stems, and has been used as an index of meristem limitation in grassland (Dalgleish and Hartnett 2006). Though drought did not affect total buds per stem, the total number of buds per stem tended to decline in grazed areas (p = 0.117, Fig. 4.10a). Pooling the entire plant community masked divergent responses of grasses, sedges, and forbs. Grazing reduced grass buds per stem from 7.5 to 5 buds per stem, approximately a 20 to 40% decline, but drought had no effect on grass buds per stem (Fig. 4.10b). In contrast, grazing had no effect on sedge and forb buds per stem, but drought reduced sedge and forb buds per stem by approximately 25% (Fig. 4.10c, d).

The Relationship Between Bud Density and Rhizome Biomass

The relationship between bud density and rhizome biomass may provide insight into how plants allocate energy under different grazing and drought conditions. Live rhizome biomass mirrored bud bank density for the entire plant community and for grasses, sedges, and forbs. I found that total live rhizome biomass did not respond to drought or grazing, with constant biomass of 1.5 to 2.5 g 0.01 m^{-2} across all treatments (Fig. 4.11a). Similarly, grass live rhizome biomass remained constant at 1 to 1.5 g 0.01 m^{-2} across grazing treatments (Fig. 4.11b). Like sedge bud and stem density, sedge live rhizome biomass was reduced by drought, with the greatest reduction under ungrazed, drought conditions (Fig. 4.11c). Neither drought nor grazing had an effect on forb live rhizome biomass (Fig. 4.11d).

I directly compared live rhizome biomass and bud densities by creating biplots of these two variables for the entire plant community, taxonomic groups of species, and for the ten most common species. I then calculate Pearson's correlation coefficient (r) and fit ranged major axis (RMA) regression models for all treatments pooled. I also fit RMA regression models for each drought by grazing combination separately to determine if drought and grazing treatments affected the relationship between bud numbers and rhizome biomass. Live rhizome biomass predicted bud density well for both the entire plant community (Fig. 4.12) and for individual plant species (Fig. 4.13). For the entire plant community, these analyses suggest that relatively less water-stressed plants generally produced more buds per unit biomass, and at a higher rate, than drought-stressed plants (Fig. 4.12a). Grasses appeared to only produce buds at a higher rate when grazed under ambient rainfall conditions (Fig. 4.12b). Sedges, however, only produced fewer buds per unit biomass when droughted and not grazed (Fig. 4.12c). Regression slopes were quite variable for forbs. A few forb species with heavy rhizomes containing secondary woody growth (e.g. *Salvia azurea* Michx. ex Lam.), produced points far below the pooled regression line and strongly influenced the forb regression fit for individual treatments (Fig. 4.12d).

Analyzing the most common plant species separately removed much of the variation and demonstrated the ability to accurately predict bud bank densities when only live rhizome biomass is known. For seven of the ten species analyzed here, Pearson's correlation (r) was greater than 0.9, and for all $r \geq 0.84$. For nearly all of the C_4 grasses (*Andropogon gerardii*, *Bouteloua curtipendula*, *Sorghastrum nutans*, and *Sporobolus compositus*), there was very little difference in the relationship between live rhizome biomass and bud density among treatments (Fig. 4.13a, b, h, i). However, *Schizachyrium scoparium* appeared to produce fewer buds per unit biomass under drought conditions than ambient conditions (Fig. 4.13g). The sedges *Carex inops* L.H. Bailey and *Carex meadii* Dewey and the C_3 grass *Dichanthelium oligosanthes* (Schult.) Gould also appeared to produce fewer buds per unit biomass under drought conditions (Fig. 4.13c, d, e). The relationship between live rhizome biomass and bud density was constant across drought and grazing treatments for the composite forb *Symphyotrichum ericoides* (L.) G.L. Nesom (Fig. 4.13j). Regression coefficients for groups and for each species are given in Table 4.2.

4.4 Discussion

Taking advantage of a severe, natural drought in addition to our drought treatment, I investigated the effects of grazing under severe drought conditions. Most vegetation responses were driven by drought, with little effect of grazing. Furthermore, drought by grazing interactions were not common. Plant community composition shifted above- and belowground in response to drought and grazing treatments. I discuss these shifts in community composition in light of the intensity of the treatment levels. I then highlight the role of bud bank in community stability, including differences in among grasses, sedges, and forbs. Sedges in particular are an under-appreciated component of tallgrass prairie plant communities, and are sensitive to both drought and grazing. Next, I consider the significance of the tight relationship between bud numbers and rhizome biomass, and implications for future bud bank research. Finally, I reflect on the potential consequences of changes in flowering and seedling survival responses versus vegetative reproduction responses.

4.4.1 Suitability of Treatment Levels

I set up precipitation interception shelters before severe drought conditions developed in 2012, giving me the opportunity to test bud bank response to grazing under severe drought conditions. The treatment levels I selected represent realistic current or future climate and grazing scenarios. Annual precipitation for 2012 was 570 mm, about 65% of the normal total. With 80% growing season interception of rainfall, drought treatments received 84 mm of rain during the growing season, and about 150 mm total for the year. Point measurements of soil moisture at the end of June indicated that soil moisture in the top 10 cm was approximately 12% in ambient plots, but 6-7% in droughted plots. While this is quite dry, during the drought of 1936 Weaver and Albertson (1936) reported less than 2% soil moisture at 2 m in a prairie near Lincoln, Nebraska for most of the growing season and the following winter. Predictions of warming temperatures and increasing drought for this region make such scenarios increasingly likely (Patricola and Cook 2013).

Similarly, clipping treatments removed 42.7±1.6% and 54.0±2.6% (mean ± SE) of ANPP in ambient and drought plots respectively, which would be considered a moderate stocking rate for this region (Towne et al. 2005). A broader range of drought and grazing treatments may reveal further nuances of vegetation responses to these disturbances, but the levels I selected are reasonable and yielded interesting insights into differing responses of grasses, sedges, and forbs.

4.4.2 Shifting Plant Community Composition

I observed shifts in plant community composition and diversity both above- and belowground within one growing season, with the strongest effects due to drought. Many earlier studies have documented that plant tolerance of drought and grazing differs among species (Tucker et al. 2011, Mullahey et al. 1990, Albertson and Weaver 1942). Large ungulate grazers generally avoid feeding on forbs in tallgrass prairie, resulting in increased relative abundance and diversity of forbs in grazed prairie (Vinton et al. 1993, Hartnett et al. 1996, Biondini et al. 1998). My results suggest that short-term forb responses to grazing depend on the relative level of water stress: forb ANPP and bud density only increases in response to grazing when moisture is available for growth. Biondini et al. (1998) also conclude that climate is the primary control over vegetation dynamics, with grazing playing a secondary role.

In some cases grazing exacerbated the effects of drought, while in other cases it did not change or ameliorated effects of drought. In areas that were both droughted and grazed, C_4 flowering stem density was completely eliminated and 45% of aboveground stems senesced between May and August. In contrast, total ANPP from grazed plots was greater than ANPP from ungrazed plots, regardless of precipitation treatment. Other studies have also documented overcompensation and increased relative growth rates in the first year of clipping tallgrass prairie grasses, but decreased productivity and relative growth rates with multiple years of defoliation (Weaver and Hougen 1939, Vinton and Hartnett 1992). Tuomi et al. (1994) demonstrated that bud sensitivity to herbivory may facilitate short-term overcompensation, but could potentially cause long-term decline in plant growth.

Some immediate vegetation responses to drought and grazing may have little effect on current-year community composition, but may strongly affect future composition. Seedlings generally contribute little to the relative abundance of any species or functional group when young. However, the regeneration niche has been identified as an important component of vegetation dynamics (Grubb 1977). Furthermore, although seedling establishment is relatively rare in tallgrass prairie (Benson and Hartnett 2006, Rogers and Hartnett 2001), even rare establishment is enough to preserve high levels of genetic diversity for clonal species, which dominate tallgrass prairie (Ellstrand and Roose 1987). Similarly, successful production of seed may not affect community composition immediately, but it may affect the trajectory of plant community recovery after drought or grazing cease (Weaver and Mueller 1942, Chapter 3).

4.4.3 Resistance to Disturbance: the Role of Bud Density and Demography

Because of their protected location beneath the soil surface, belowground buds are positioned to play a significant role in plant community resistance to damage by drought and grazing. Large ungulate grazers preferentially feed on grass in tallgrass prairie (e.g. Towne et al. 2005), and grasses tolerate grazing by placing their meristems at or below the soil surface, out of the reach of grazers. Grass stem density generally increases in response to grazing as more buds are released to grow into aboveground tillers, but the number of buds per stem may remain constant (N'Guessan and Hartnett 2011), or declines (Mullahey et al. 1990, 1991, this study).

I earlier posed alternative hypotheses that the combination of drought and grazing might cause increased, similar, or decreased bud bank density relative to drought or grazing treatments applied separately. Unlike my previous study (Chapter 2), I found that drought reduced total bud bank density regardless of grazing treatment, although the responses of grasses, sedges, and forbs were idiosyncratic. This suggests that the response of the bud bank depends largely on the relative abundance of different groups of species. In Chapter 2, sedge and forb buds accounted for 15-20% of total buds, while in this study sedge and forb buds accounted for 10-35% of total buds. Because sedges and forbs responded more strongly to drought and grazing, greater relative abundance of these groups could lead to greater changes in the vegetation. In the current study I found that even after imposing drought and grazing treatments in a year of low ambient precipitation and record heat, grass bud bank density did not decline, highlighting their role in tallgrass prairie resistance to pulsed disturbances.

The ratio of buds per stems I observed in response to drought and grazing agreed with previous reports. In Chapter 2 I found that the overall number of buds per stem varied by year, not by precipitation treatment, ranging from 7.5 to 9.5 in years with fire, and from 6 to 7.5 in years without fire. In Chapter 3 I found that grazing reduced the number of buds per stem for grasses by 15-25% in 3 of the 4 years observed, and Mullahey et al. (1991) also found that defoliation reduced bud production of *Calamovilfa longifolia* (Hook.) Scribn. and *Andropogon hallii* Hack. My study demonstrates that grazing reduces the number of buds per stem approximately 25% under both drought and ambient precipitation. Thus stems that emerge in response to grazing do not appear to produce buds at similar rates to ungrazed tillers. Since bud production per unit biomass is stable for C_4 grasses under under drought and grazing, bud production per tiller in C_4 grasses may be depressed because fewer phytomers, and thus fewer buds, are produced per tiller. However, both N'Guessan and Hartnett (2011) and Mullahey et al. (1990) found that grazing did not reduce bud production by *Schizachyrium scoparium*.

4.4.4 Sedges, an Under-Studied Component of Tallgrass Prairie

I found high abundance of the sedges *Carex meadii* and *Carex inops* across all treatments. With 33 species known, Cyperaceae is the fourth most abundant family at Konza Prairie Biological Station, but is rarely studied compared to the most abundant families, Poaceae, Asteraceae, and Fabaceae (Towne 2002). Sedges are often lumped with cool-season C_3 grasses even though some sedges use the C_4 pathway (Besnard et al. 2009) and may respond differently to fire and grazing (Towne et al. 2005, Towne and Kemp 2008). The tradition of annual spring burning in the Flint Hills of Kansas appears to reduce the abundance of sedges (Towne and Kemp 2008). While dominance of warm-season grasses drives ecosystem function in tallgrass prairie, subdominant species, including sedges, account for most of the plant taxonomic diversity in tallgrass prairie. A diverse plant community is necessary for survival of organisms of higher trophic levels (Stoner and Joern 2004).

Sedges may be an important source of high-quality forage for bison and cattle when warm-season grasses are not growing (Courant and Fortin 2010, Volesky et al. 2005). In Oklahoma tallgrass prairie sedges are selectively grazed by bison (Coppedge et al. 1998). Towne et al. (2005) found that in contrast to C_4 grass abundance, sedge abundance increased with grazing by cattle and bison in annually burned pastures. My results suggest that sedges are resistant to defoliation by grazers, but sensitive to drought. Because of their abundance and potential importance for vegetation response to common disturbances in tallgrass prairie, the role of Cyperaceae in this system needs further study.

4.4.5 Relationship between Rhizome Biomass and Bud Numbers

The relationship between rhizome biomass and bud numbers has large practical and theoretical implications. First, various methods have been proposed for estimating bud density. Direct counts of buds, the method I employed, has been called “very time consuming” (Klimešová and Klimeš 2007), which may be an underestimate of the amount of time required to determine bud densities (personal observation). Klimešová and Klimeš (2007) also suggest counting the number of nodes on rhizomes, but this method may be inaccurate if the nodes on the neck of a rhizome in grasses and sedges, which do not produce buds (Liese 1998), are counted. Furthermore, estimates of bud density may vary by observer, depending on use of microscopes, knowledge of species morphology, and experience. The excellent correlation between bud density and live rhizome biomass suggests a new method for estimating bud densities that may allow better comparisons among observers, studies, and species. If bud densities are desired, relationship between bud density and rhizome biomass could be determined with a subset of samples, and used to calculate bud densities for the remaining samples. Even if bud numbers are questionable, it would be easier to compare among studies if investigators reported live rhizome biomass in addition to bud densities. The additional work required to dry and weigh the rhizomes would place little additional burden of time and resources on investigators, and easily outweighed the potential costs from using this informative metric. Among species, the slope of the regression line (buds per unit biomass) is potentially a very useful clonal plant trait across a range of species.

I found that the number of buds per gram of biomass did not vary among treatments for some species, but did vary for other species. Many of the C_4 grasses produced buds at a similar rate regardless of drought and grazing treatments. However, *Dichanthelium oligosanthes* and *Schizachyrium scoparium* tended to produce more buds per unit per unit biomass under ambient precipitation relative to droughted plants. These two caespitose, or bunchgrasses are known to display higher order bud production, which is production of additional buds on primary buds before they elongate and emerge as aboveground stems (Ott and Hartnett 2012, Cable 1971). Higher order bud production may represent a low-cost bet hedging strategy to buffer plants from future disturbance. Where bud production rate varies by treatment, this many indicate plasticity in plant development that may have important consequences for species responses to disturbance.

4.5 Conclusions

After one growing season, most vegetation responses to short-term disturbance treatments were driven by drought. While grazing did effect some responses, particularly ANPP and grass buds per stem, it had no effect on most other responses. Furthermore, I observed few drought by grazing interactions. I therefore found some support for my hypothesis that grazing during drought may have little additional effects on vegetation.

Previous workers have suggested that bud banks are an under-appreciated component of vegetation dynamics, and are potentially useful for helping explain variation in ANPP across a precipitation (Dalgleish and Hartnett 2006) and play an important role in plant community recovery from disturbance (Rogers and Hartnett 2001, Klimešová 2007). The relatively small response of the grass bud bank density to drought and grazing suggests that this belowground reservoir of propagules mediates the resistance and resilience of tallgrass prairie vegetation to disturbance. Grasses may prioritize production of new buds before allocating energy to further aboveground growth or sexual reproduction. Variation in species responses to drought and grazing may increase overall plant community stability. The phenology of growth and bud production, and coincidence with the timing of drought and grazing, may determine plant species' responses to these disturbances.

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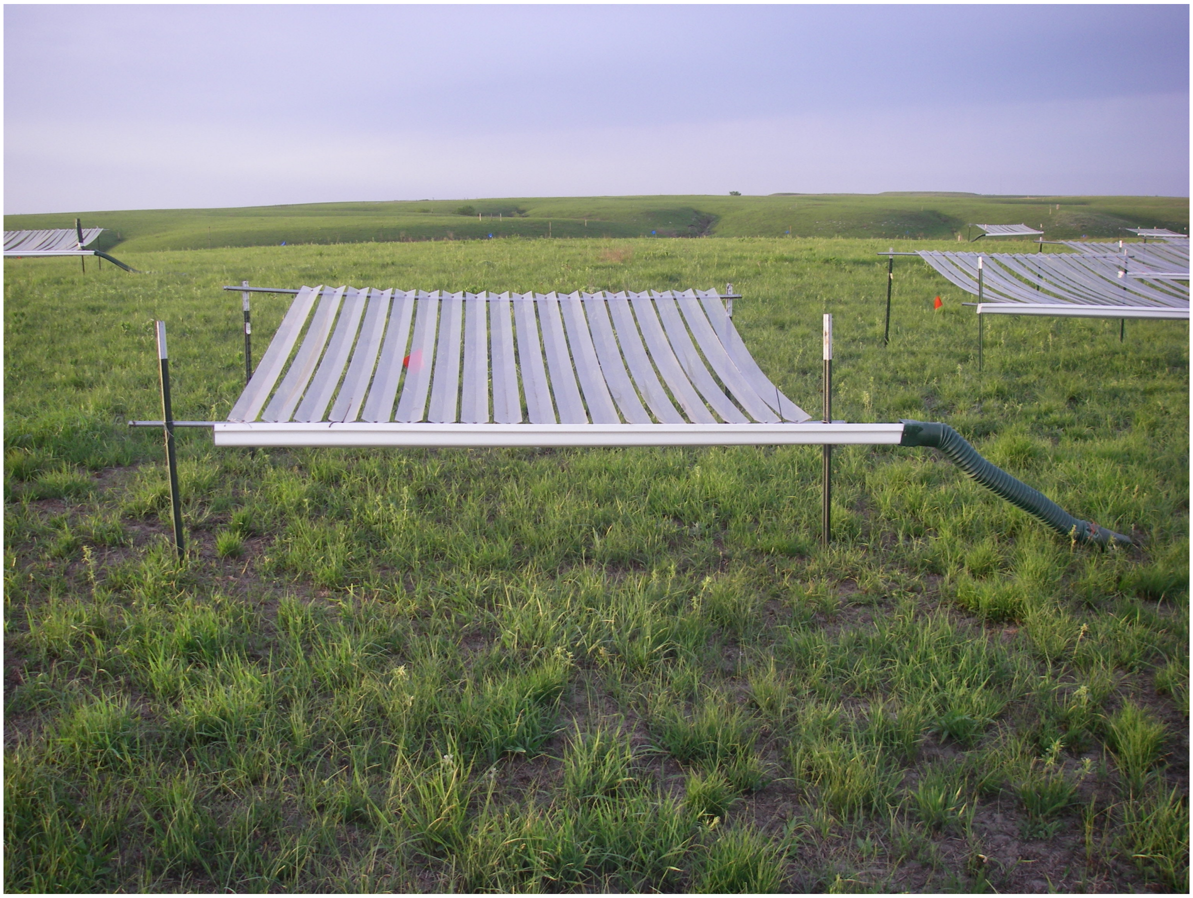

Figure 4.1: A 2.4 × 2.4 m rainfall interception shelter, with shingles spaced to cover 80% of the total shelter area. Picture taken 26 April 2012 by B. VanderWeide.

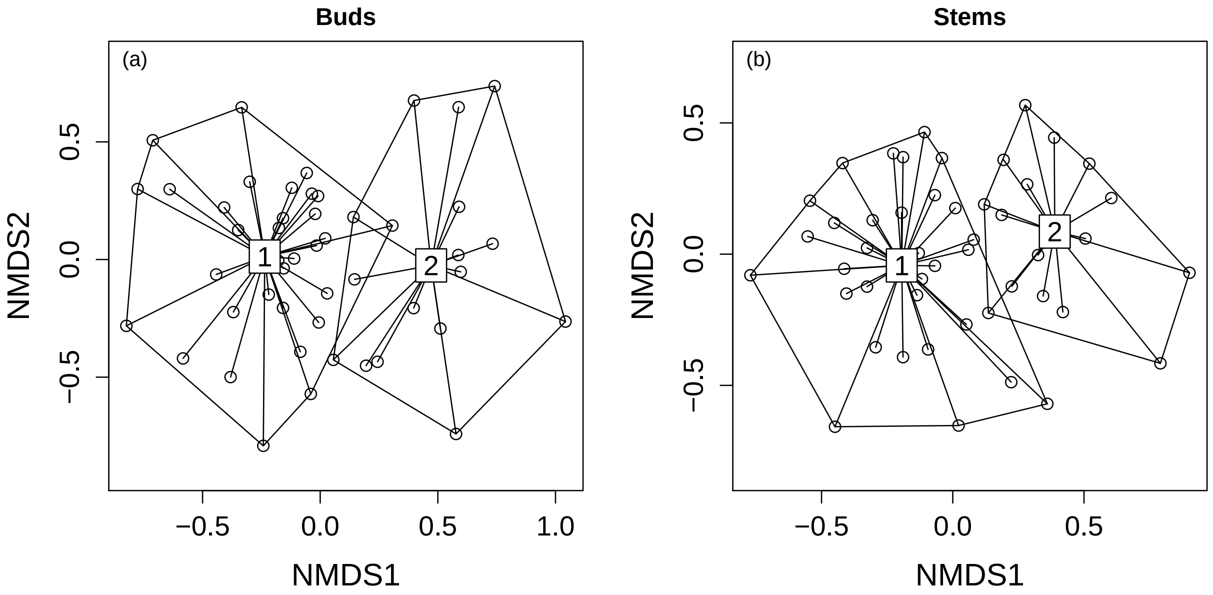

Figure 4.2: Non-metric multi-dimensional scaling (NMDS) ordination on (a) buds and (b) stems to confirm blocking plots by location. These blocks were then accounted for when assessing changes in plant species composition due to drought and grazing treatments. Each point represents a plot ($N = 48$).

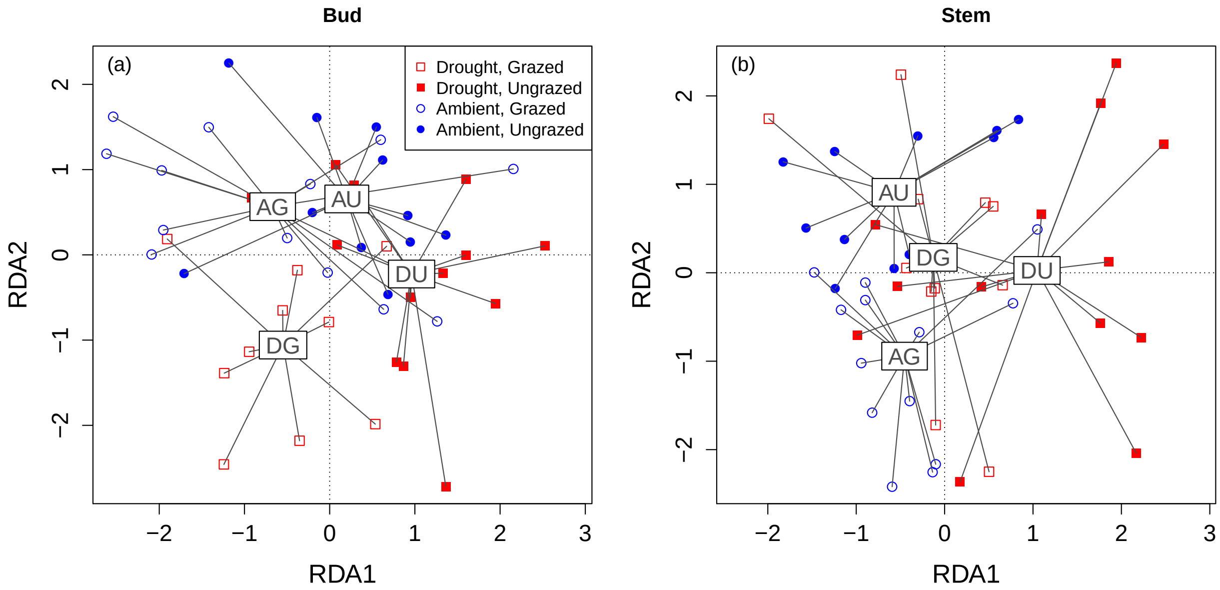

Figure 4.3: Distance-based redundancy analysis (db-RDA) on (a) buds and (b) stems. Points represent individual plots, and lines connect plots to the treatment centroid (AU = ambient, ungrazed; AG = ambient, grazed; DU = drought, ungrazed; DG = drought, grazed). Each point represents a plot.

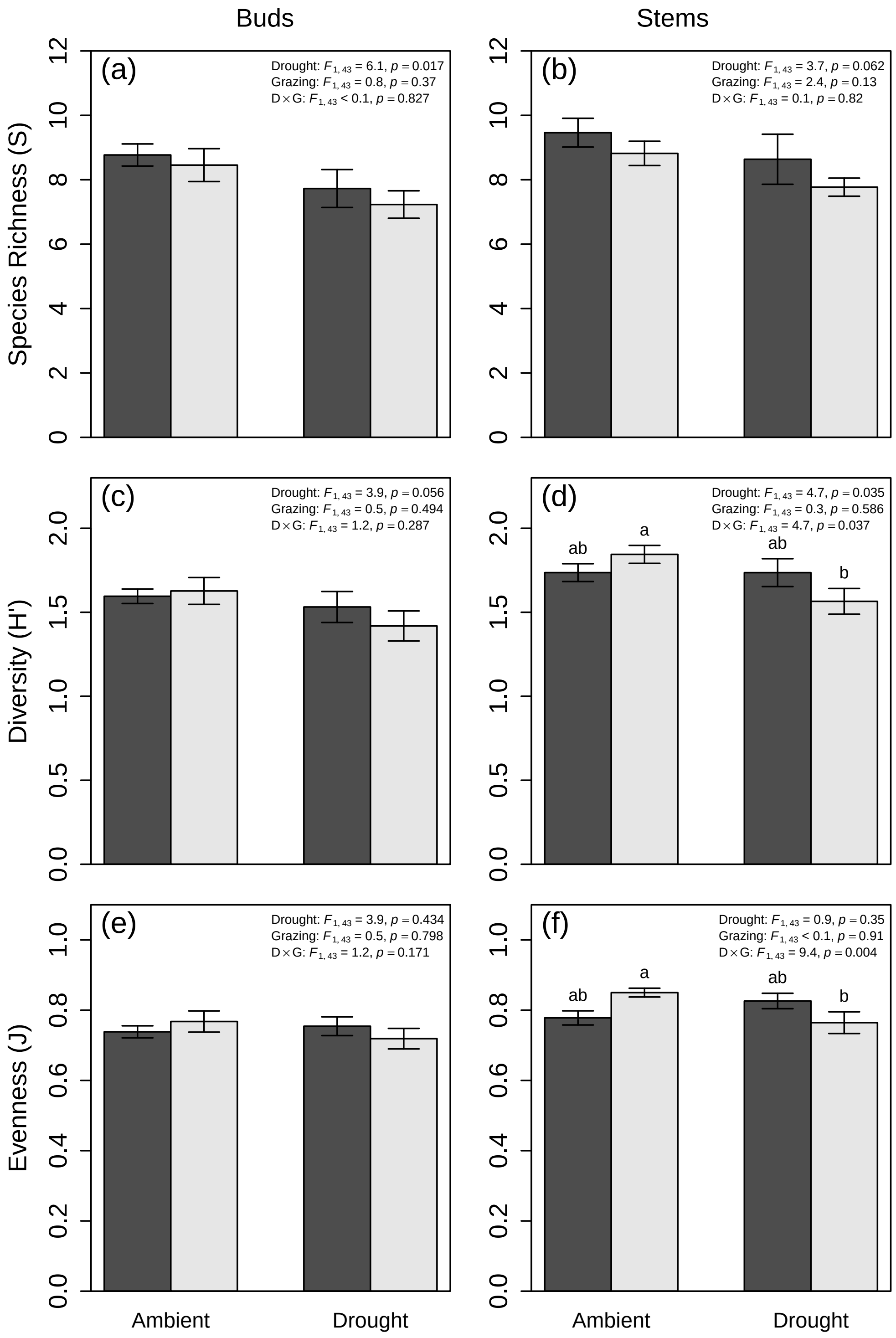

Figure 4.4: The response of species richness (a, b), Shannon diversity (c, d), and evenness (e, f) in response to drought and grazing treatments. The belowground (bud bank density) response is shown in the left column, and the aboveground (peak stem density) response in the right column. Light shaded bars are ungrazed, dark bars are grazed. Bars are means ± 1 SE. Lowercase letters above error bars indicate significant differences among all treatment combinations.

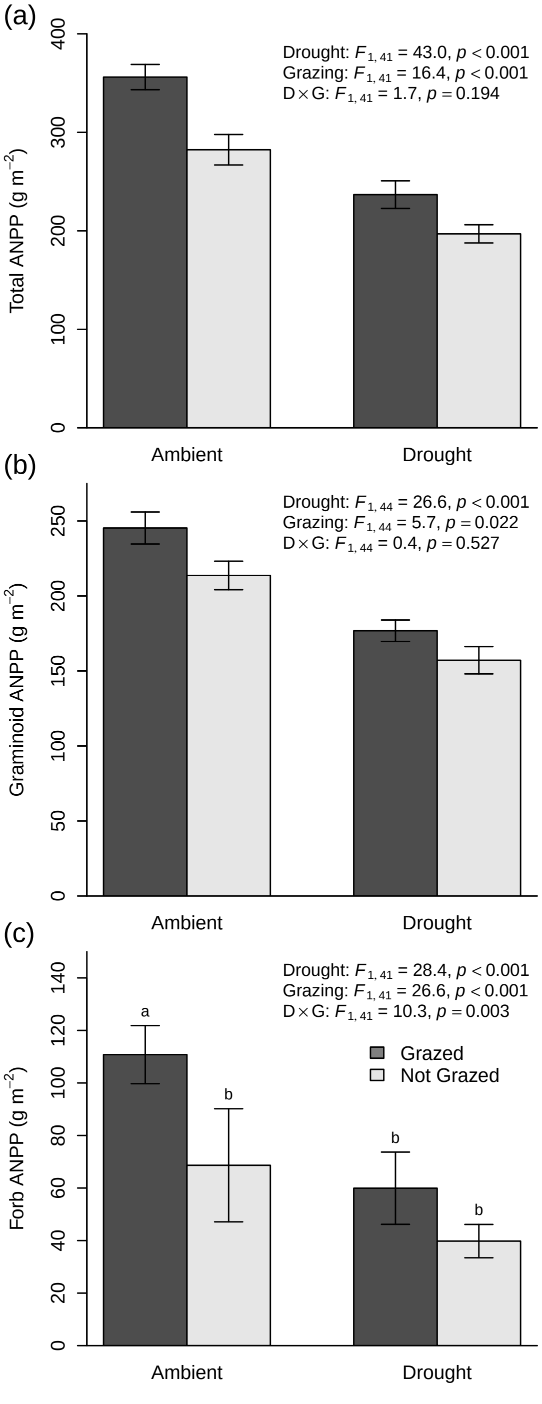

Figure 4.5: Response of aboveground net primary productivity (ANPP) to drought and grazing treatments. Dark shaded boxes are grazed, and light shaded boxes are ungrazed. Analysis shown for forb ANPP was done without three outlier points (ambient, not grazed = 269.63 g m^{-2}; ambient, grazed = 196.16 g m^{-2}; drought, grazed = 192.26 g m^{-2}), caused by presence of large clonal forbs or woody shrubs on the plots. When included in the analysis, drought and clipping effects remain significant ($p < 0.01$), while the interaction is not ($p = 0.194$). Bars are means ± 1 SE. Lowercase letters above error bars indicate significant differences among all treatment combinations.

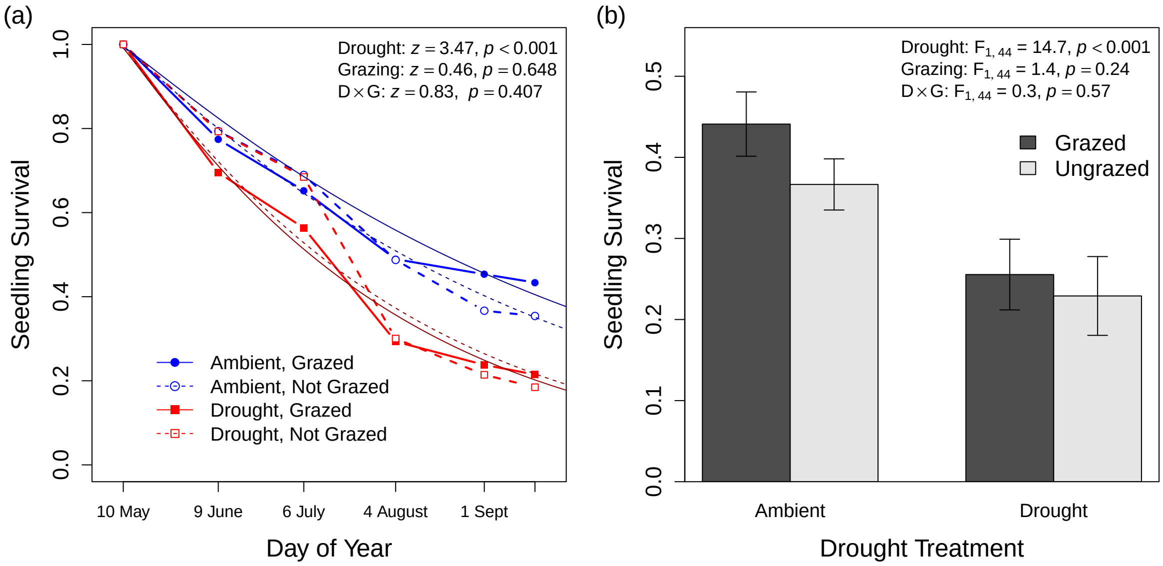

Figure 4.6: Seedling survival in response to drought and grazing treatments (a) throughout the growing season and (b) at the end of the growing season. Curves in (a) represent the fitted parametric regression model, with colors and line types following the legend. Bars in (b) represent the mean ± 1 SE.

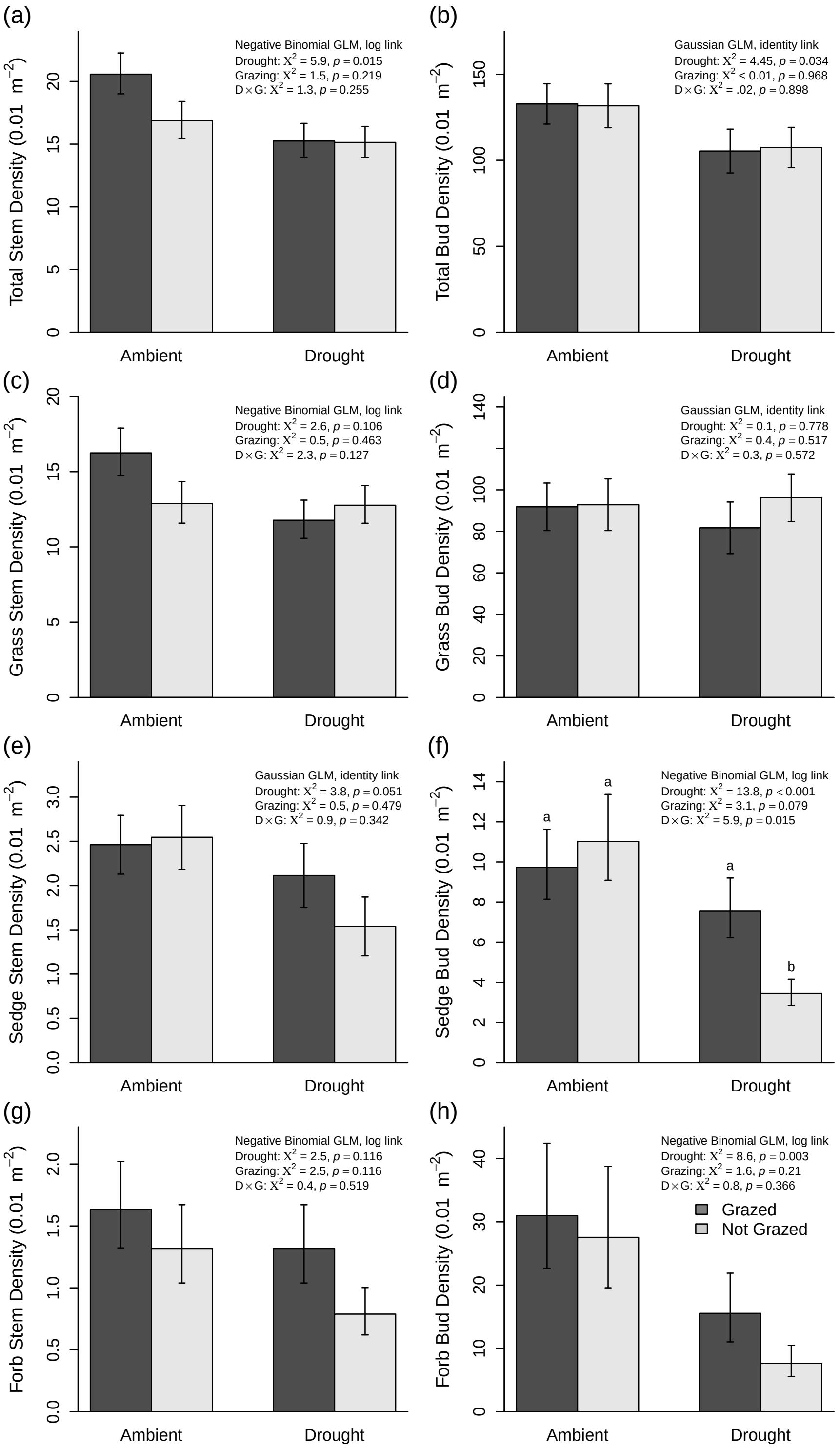

Figure 4.7: Densities of stems (a,c,e,g) and buds (b,d,f,h) directly above and in 0.01 m^2 soil cores for (a, b) the entire plant community, (c,d) grasses, (e,f) sedges, and (g,h) forbs. All χ^2 tests have one degree of freedom. Bars are means ± 1 SE. Lowercase letters above error bars indicate significant differences among all treatment combinations.

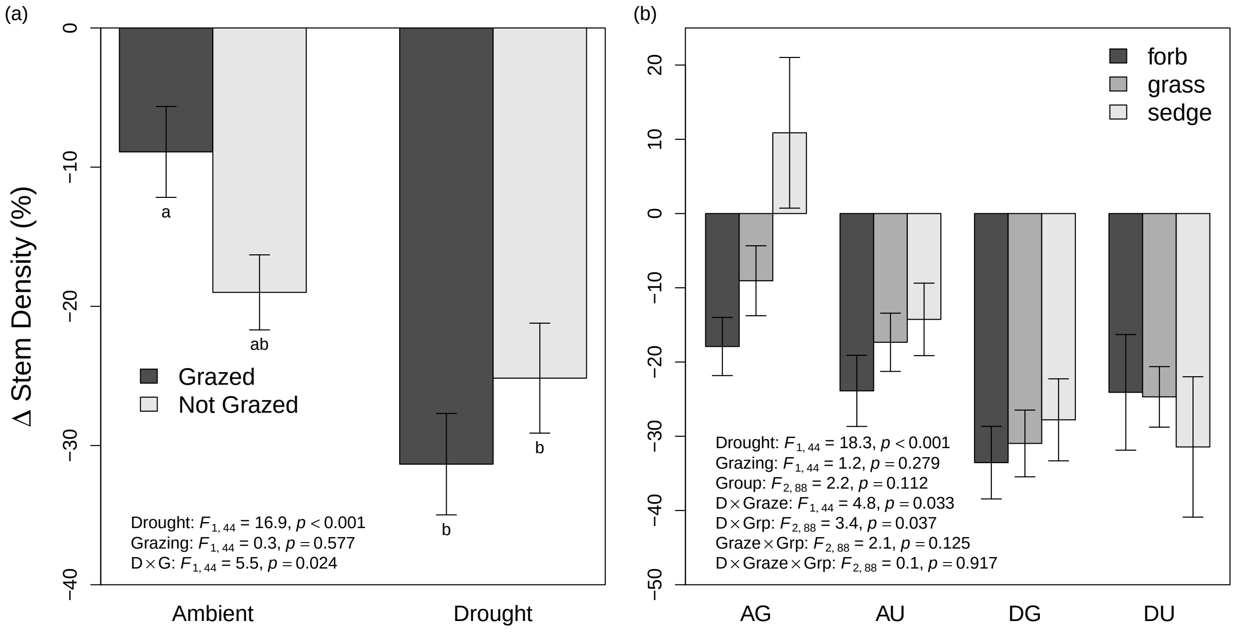

Figure 4.8: Change in stem density from May to August, as a percent of May stem density. Bars are means ± 1 SE. In (b), AG = ambient, grazed; AU = ambient, ungrazed; DG = drought, grazed; and DU = drought, ungrazed. Bars are means ± 1 SE. Lowercase letters above error bars indicate significant differences among all treatment combinations.

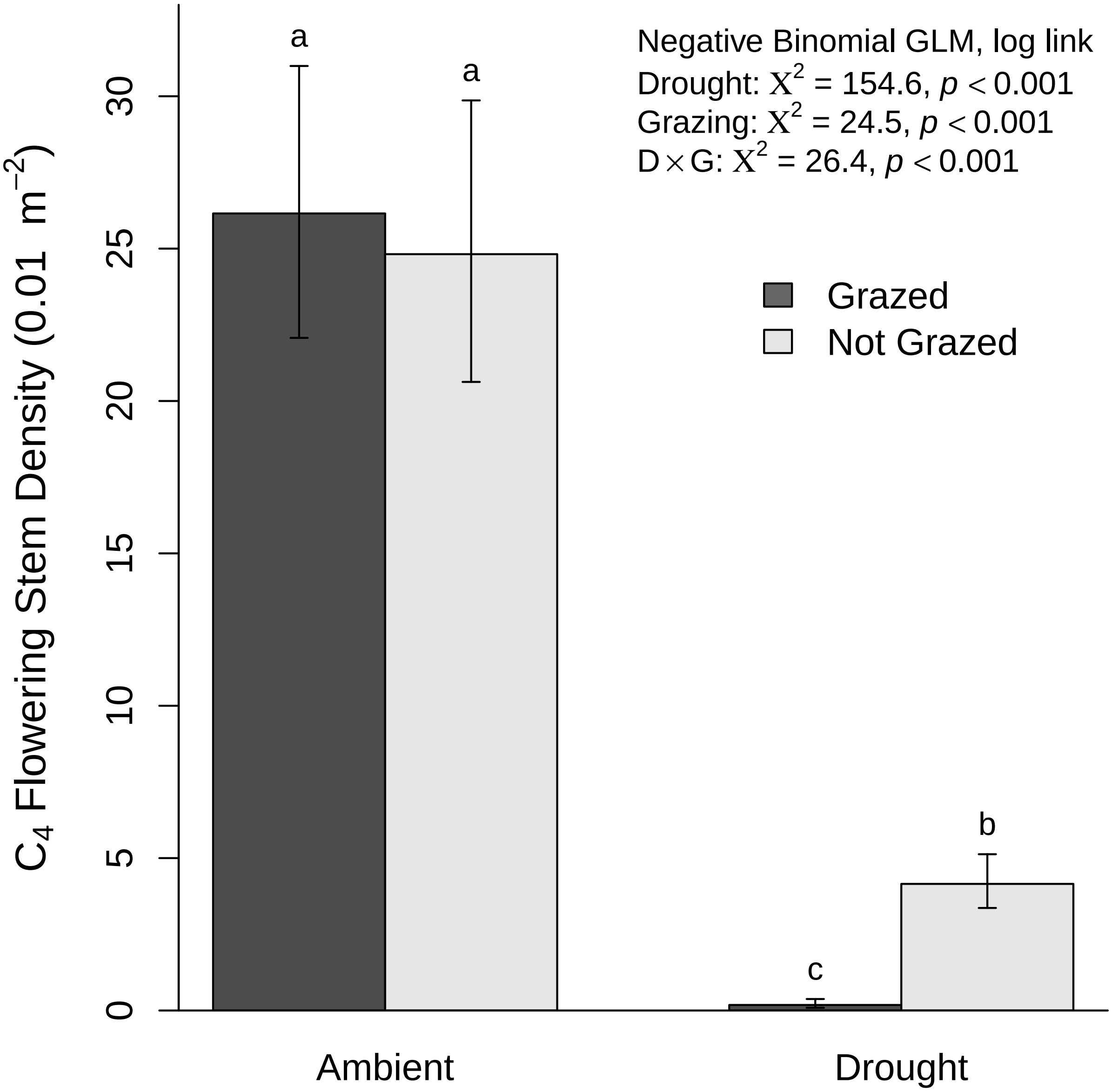

Figure 4.9: Density of C_4 grass flowering stems in response to drought and grazing treatments. Bars are means ± 1 SE. Lowercase letters above error bars indicate significant differences among all treatment combinations.

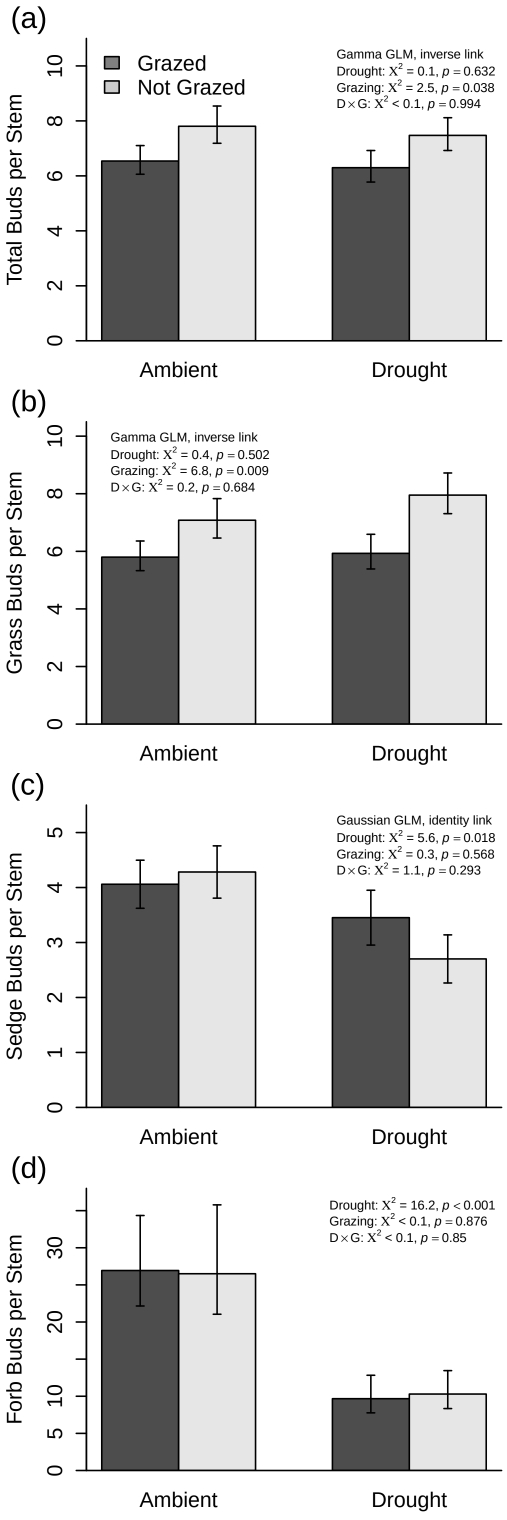

Figure 4.10: Buds per stem for (a) the entire plant community, (b) grasses, (c) sedges, and (d) forbs. Bars are means ± 1 SE.

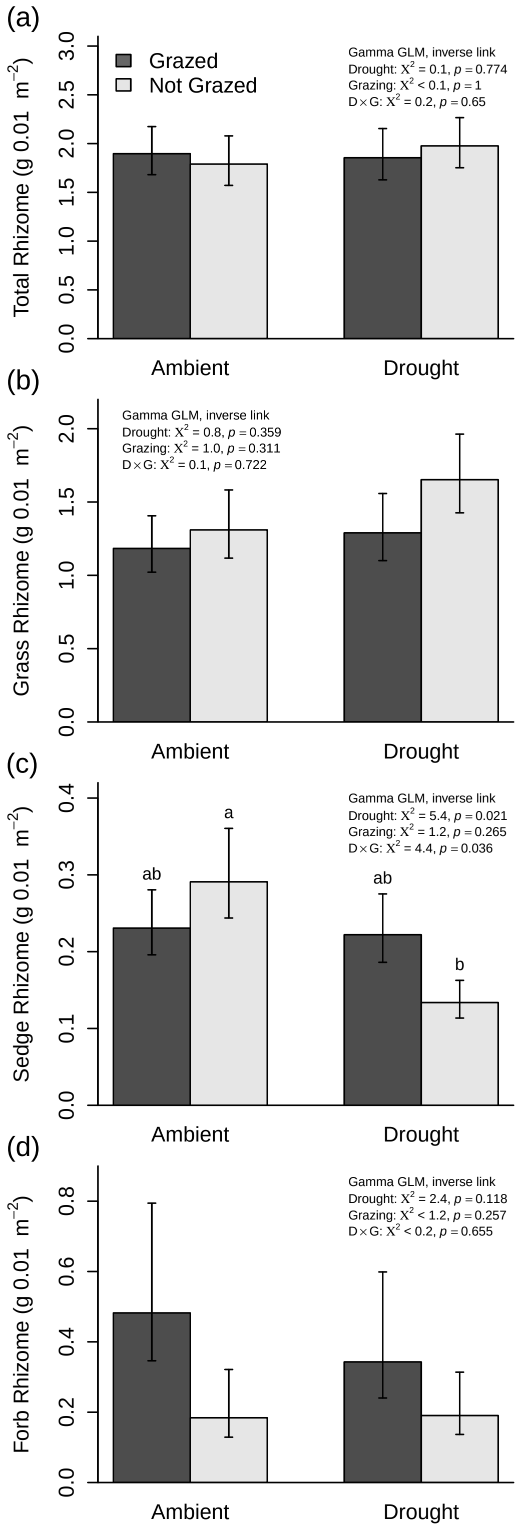

Figure 4.11: Belowground live rhizome biomass, estimated in the 0.01 m^2 soil cores used for bud density estimates. Biomass is summarized by (a) the entire plant community, (b) grasses, (c) sedges, and (d) forbs. Bars are means ± 1 SE. Lowercase letters above error bars indicate significant differences among all treatment combinations.

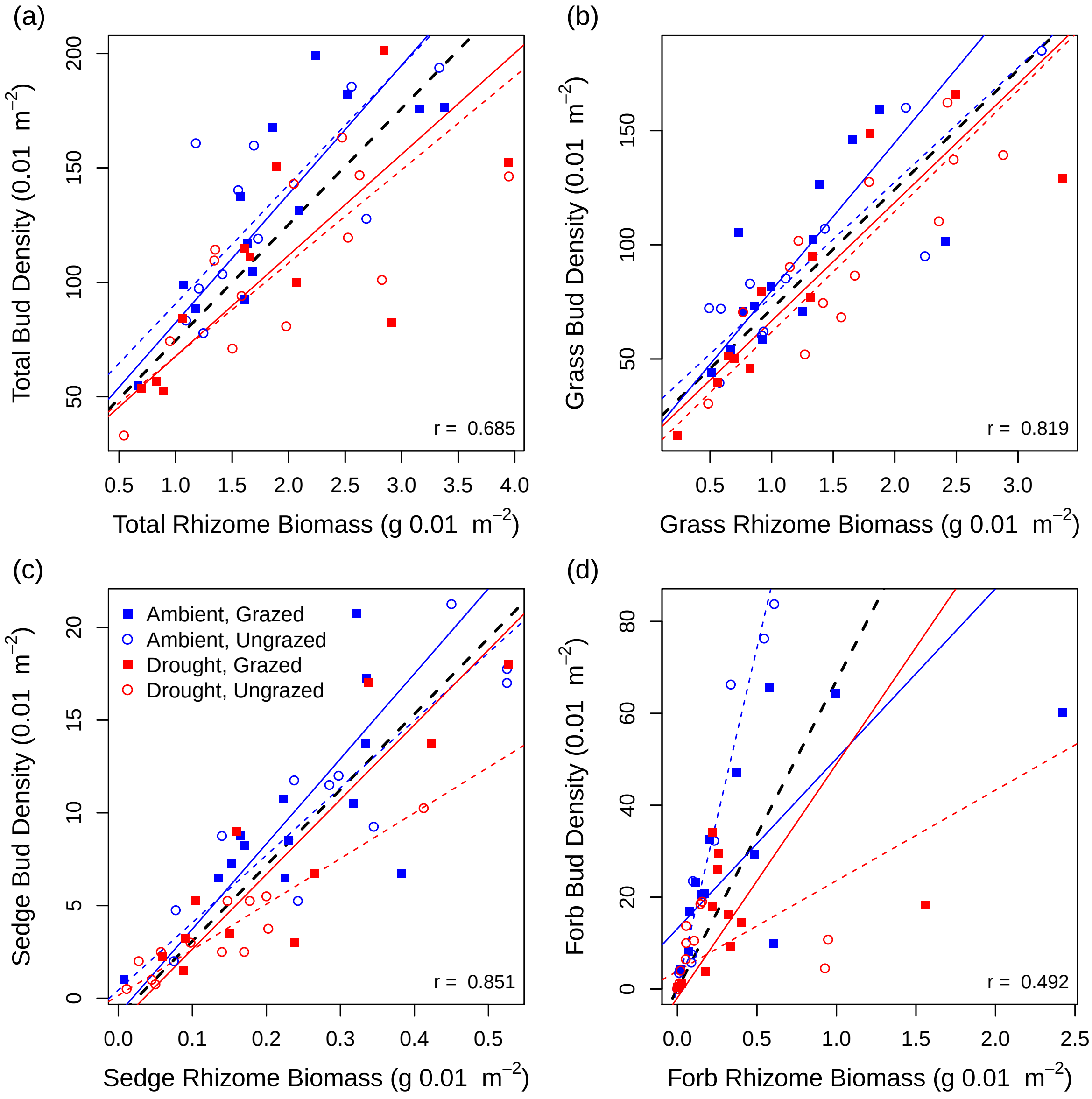

Figure 4.12: Relationship between bud density and rhizome biomass for (a) the entire plant community, (b) grasses, (c) sedges, and (d) forbs. Lines are the ranged major axis (RMA) regression fit for all data points (black, dashed), ambient and grazed (blue solid), ambient and ungrazed (blue dashed), drought and grazed (red solid), and drought and ungrazed (red dashed). Pearson's correlation (r) for all treatments pooled is given. Regression coefficients for pooled treatments (black, dashed line) is included in Table 4.2.

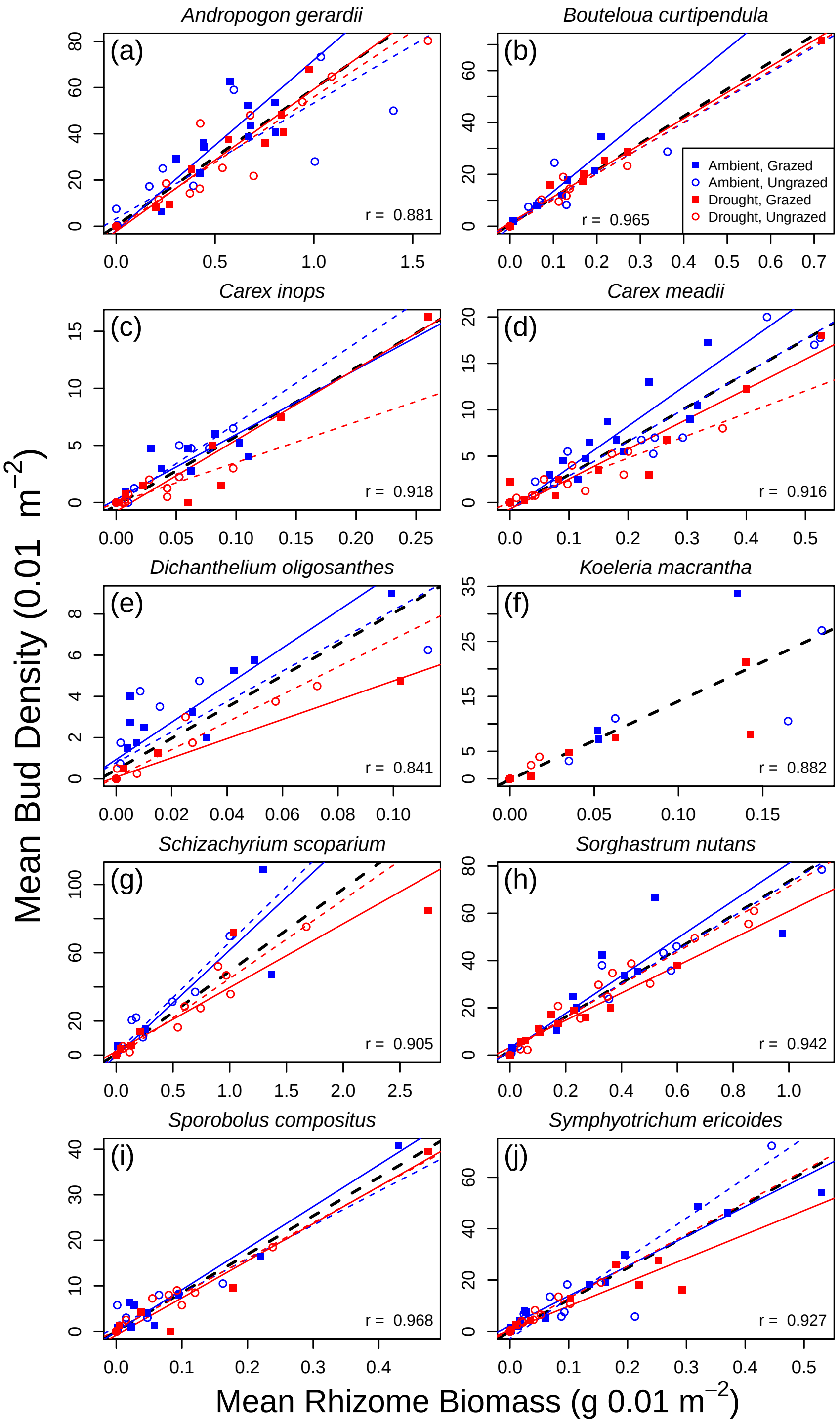

Figure 4.13: Relationship between bud density and rhizome biomass for the 10 species encountered in at least 10 plots. Lines are the ranged major axis (RMA) regression fit for all data points (black, dashed), ambient and grazed (blue solid), ambient and ungrazed (blue dashed), drought and grazed (red solid), and drought and ungrazed (red dashed). Regression models were not fit by treatment for *Koeleria macrantha* because of small sample sizes. Pearson's correlation (*r*) is given for pooled treatments. Regression coefficients for pooled treatments (black, dashed line) is included in Table 4.2.

Table 4.1: Parametric regression analysis of the effect of drought and grazing treatments of seedling survival. Fitted lines are plotted in Fig. 4.6a, with parts of this table reproduced for convenience. The "Value" column indicates regression coefficients. The significant, positive coefficient for drought indicates that seedling in ambient plots survive longer than seedling in drought plots.

	Value	Std. Err	z	p
(Intercept)	4.6795	0.0501	93.49	< 0.001
drought	0.2214	0.0638	3.47	0.001
grazing	0.0238	0.0521	0.46	0.648
drought×grazing	0.0446	0.0539	0.83	0.407
Log(scale)	-0.0698	0.2008	-0.347	0.728
Scale (reciprocal of Wiebull shape parameter) = 0.933				

Table 4.2: Ranged major axis (RMA) regression coefficients and confidence intervals for the relationship between mean bud density of four 0.01 m^2 soil cores per plot and the mean live rhizome biomass (g) in the same cores. The upper and lower estimates of the 95% confidence intervals for the intercept and slope are indicated by 2.5% and 97.5%. The correlation coefficient (r, Pearson's product-moment correlation) was significant at $p < 0.001$ for all groups and species.

	Intercept	2.5%	97.5%	Slope	2.5%	97.5%	r
Total	23.73	-12.61	50.09	50.76	36.77	70.05	0.685
Grass	19.62	3.06	33.19	52.29	42.34	64.44	0.819
Sedge	-1.00	-2.79	0.49	40.82	33.96	49.10	0.851
Forb	0.01	-18.96	7.68	67.07	41.76	129.69	0.492
Andropogon gerardii	0.29	-4.69	4.43	59.22	50.54	69.66	0.881
Bouteloua curtipendula	0.69	-0.02	1.35	104.27	96.18	113.07	0.965
Carex inops	-0.25	-0.57	0.03	60.33	53.02	68.61	0.918
Carex meadii	-0.65	-1.51	0.11	36.49	32.02	41.55	0.916
Dichanthelium oligosanthes	0.44	0.19	0.66	75.97	62.60	92.03	0.841
Koeleria macrantha	-0.18	-0.74	0.31	143.03	121.59	167.24	0.882
Schizachyrium scoparium	0.96	-1.56	3.10	48.17	41.94	55.53	0.905
Sorghastrum nutans	1.82	-0.52	3.92	71.70	64.48	79.75	0.942
Sporobolus compositus	0.05	-0.35	0.41	84.41	78.10	91.22	0.968
Symphyotrichum ericoides	-0.09	-1.58	1.24	124.09	109.95	139.87	0.927

Table 4.3: Summary of the effects of drought and grazing on tallgrass prairie vegetation. Arrows pointing up indicate that a response increased, arrows pointing down indicate that a response decreased, and (·) indicates no difference in response between levels of drought or between levels grazing. An asterisk (*) indicates a significant change in community composition ($p < 0.1$). If a significant interaction between drought and grazing was observed, it is indicated with “yes.”

		Drought	Grazing	Interaction?
Species Composition	Aboveground	*	·	·
	Belowground	·	*	·
S	Aboveground	⇓	·	·
	Belowground	⇓	·	·
H'	Aboveground	⇓	·	yes
	Belowground	⇓	·	·
ANPP	Total	⇓	⇑	yes
	Graminoid	⇓	⇑	·
	Forb	⇓	⇑	yes
Rhizome Biomass	Total	·	·	·
	Grass	·	·	·
	Sedge	⇓	·	yes
	Forb	·	·	·
Bud Density	Total	⇓	·	·
	Grass	·	·	·
	Sedge	⇓	·	yes
	Forb	⇓	·	·
Stem Density	Total	⇓	·	·
	Grass	·	·	·
	Sedge	⇓	·	·
	Forb	·	·	·
Buds per Stem	Total	·	·	·
	Grass	·	⇓	·
	Sedge	⇓	·	·
	Forb	⇓	·	·
C_4 Flowering		⇓	⇓	yes
Seedling Survival		⇓	·	·

Chapter 5

New insights on the role of bud banks in vegetation dynamics of tallgrass prairie

The mantra of plant population biologists, particularly the intellectual descendents of John Harper, states that we can understand plant responses to their environments by studying birth, survival, and death rates of plant parts (Harper 1977). This approach is particularly useful for clonal plants because it is difficult to identify genetically distinct individuals (genets). Sessile plants respond to the environment by altering birth and death rates of population of their parts, for example leaves, stems, and roots. The study of bud bank demography is a direct extension of John Harper's view of the world: by understanding the processes that control the birth, death and survival rates of buds, we might gain insight into the mechanisms that drive patterns and processes in communities and ecosystems. But why buds? Because these belowground propagules are the source of nearly all aboveground stems in tallgrass prairie.

However, even when studying populations of parts, we must remember the context of the whole. The conceptual diagrams I presented in Chapter 1 attempt to place bud demography in the context of the annual cycle of perennial plant growth (Figs. 1.1 and 1.2). Axillary buds are produced with the growth of new phytomers, the module that includes the internode, node, leaf, and axillary bud. Buds are lost from the bud bank through mortality (resulting from senescence, predation, or disease) or growth into a stem. In the discussion below and in Table 5.1, I briefly compare and contrast results of the previous chapters in the context of this annual cycle. I then highlight new insights from these studies and discuss potential future directions for bud bank research.

5.1 Comparison of Studies

Bud bank densities resisted perturbation from pulsed changes in precipitation or grazing regimes, but persistent disturbance or extreme events caused changes in bud bank density. Irrigation led to increased bud bank density, but only after the second year of increased water availability (Chapter 2). Similarly, long-term grazing reduced bud bank density by increasing initiation of new graminoid stems but decreasing the number of buds produced per stem (Chapter 3). Short-term extreme drought reduced bud bank density through its effects on sedges and forbs, while grasses resisted changes from extreme drought (Chapter 4).

Changes in ANPP were generally not reflected in the bud bank, suggesting that production of buds and productivity are partially decoupled. When I observed strong reductions in ANPP in response to drought, or increased ANPP in response to grazing, I did not observe similar changes in bud bank density. Likewise, short-term changes in bud bank demography in response to fire or other disturbances did not immediately change total bud bank density. Because the buds of the dominant C_4 grasses persist in the soil for over a year (Ott and Hartnett 2012), they may induce a storage effect, buffering the plant community against short-term variation in resource availability. The pre-disturbance composition of the plant community, and particularly the relative abundances of forbs, sedges, and C_4 grasses, may affect the immediate vegetation response to disturbance.

Plant community composition and diversity shifted in response to both drought and grazing. When drought is severe, but not extreme (Chapter 2), it may primarily cause shifts in species' abundance; extreme drought, however, may change plant community composition by increasing species turnover (Chapter 4). While long-term grazing increased species richness and diversity (Chapter 3), it did not increase forb bud bank density, biomass (Chapter 3), or rates of seedling survival (Chapter 4). Instead, short rest periods from grazing appeared to be important for increasing forb biomass and seed bank density (Chapter 3). Increased diversity with grazing may not be the result of increased seedling survival. Increased light penetration to the soil surface, greater variation in soil temperature, and increased nutrient availability may cue more seedlings to germinate, resulting in greater absolute recruitment into larger size classes, though perhaps at the same rate.

5.2 New Insights and Future Directions

1. **Limitations of using density to estimate bud bank demographic rates.** While the research presented here increased our understanding of bud bank dynamics in tallgrass prairie, it was not possible to determine exact causes of change in the bud bank because I measured changes in total bud bank density. Various scenarios could account for changes in bud bank density, including change in bud natality, survival, mortality, or growth into aboveground stems. Increased bud bank density could be caused by normal bud production but decreased losses from mortality and stem growth, or, alternatively, from increased bud production and normal rates of attrition. A fruitful, yet challenging avenue of future research would investigate changes in inter-annual bud natality, survival, and mortality rates under different disturbance regimes. One possible approach would be to label buds and rhizomes with isotopes, and use differences in abundance of the isotope to determine the age of buds and rhizomes. This would provide estimates of longevity of these belowground parts of perennial, herbaceous plants and contribute to our understanding of their role in vegetation resistance and resilience.

2. **Stem density $\ll$ bud bank density**. This research clearly demonstrates that the number of buds greatly exceeds the number of stems. Across years and disturbance regimes, only 5 - 25% of belowground buds were cued to emerge as aboveground stems. Therefore, meristem limitation is very unlikely in tallgrass prairie. However, changes in species abundance aboveground may be partially explained by inter-specific changes in rates of bud growth into stems in response to disturbance.

3. **Bud number and live rhizome biomass are highly correlated.** Within species, bud density is highly correlated with live rhizome biomass (Chapter 4). Using rhizome biomass as a proxy for bud density may allow studies of bud bank dynamics across broader spatial and temporal scales. Furthermore, less training is required to determine rhizome weight than

bud counts, and rhizome density may be less to biased by experience and motivation level of individual observers than bud counts.

4. **Plant phenology and disturbance timing**. The phenology of plant growth and the timing of disturbance may be very important for determining population and community responses to disturbance. Ott and Hartnett (2012) described the phenology of bud production by the C_4 grass *Andropogon gerardii* and the C_3 grass *Dichanthelium oligosanthes*, which I have redrawn in Figure 5.1. Bud production of *A. gerardii* begins as soil temperatures warm in the spring, and 75% of buds are produced in one month. Bud production slows well before soil moisture becomes limiting in 2008. In contrast, most buds of *D. oligosanthes* are produced in the fall as temperatures cool and soil moisture recovers. These two species appear to time their bud production during periods when temperatures are favorable for growth and water is historically available. Is disturbance during periods of bud production relatively more damaging than disturbance at other times of year?

5. **Grazing recovery and patch-burn grazing**. Conservation scientists have recently begun to advocate patch-burn grazing. This system divides a pasture into sections, with one section burned per year on a rotating schedule. The cattle preferentially graze the burned areas, allowing unburned areas to rest. Instead of favoring species that do well under a homogeneous disturbance regime, patch-burn grazing promotes persistence of a broader biota by creating of mosaic of habitat that varies in time since burning and grazing pressure. This management strategy may not only be good for grassland fauna, but also for sub-dominant plant species. My research indicates that forb abundance increases during the years immediately after grazers are removed Chapter 3. This results in greater seed bank density relative to areas that continue to be grazed. Despite the increased vigor of grasses, sub-dominant perennial plant species that were able to establish during grazing persist because of long-lived rhizomes and buds. Patch-burn grazing mimics the disturbance regime applied in this study, and may therefore increase species diversity in both the above- and belowground parts of the plant community.

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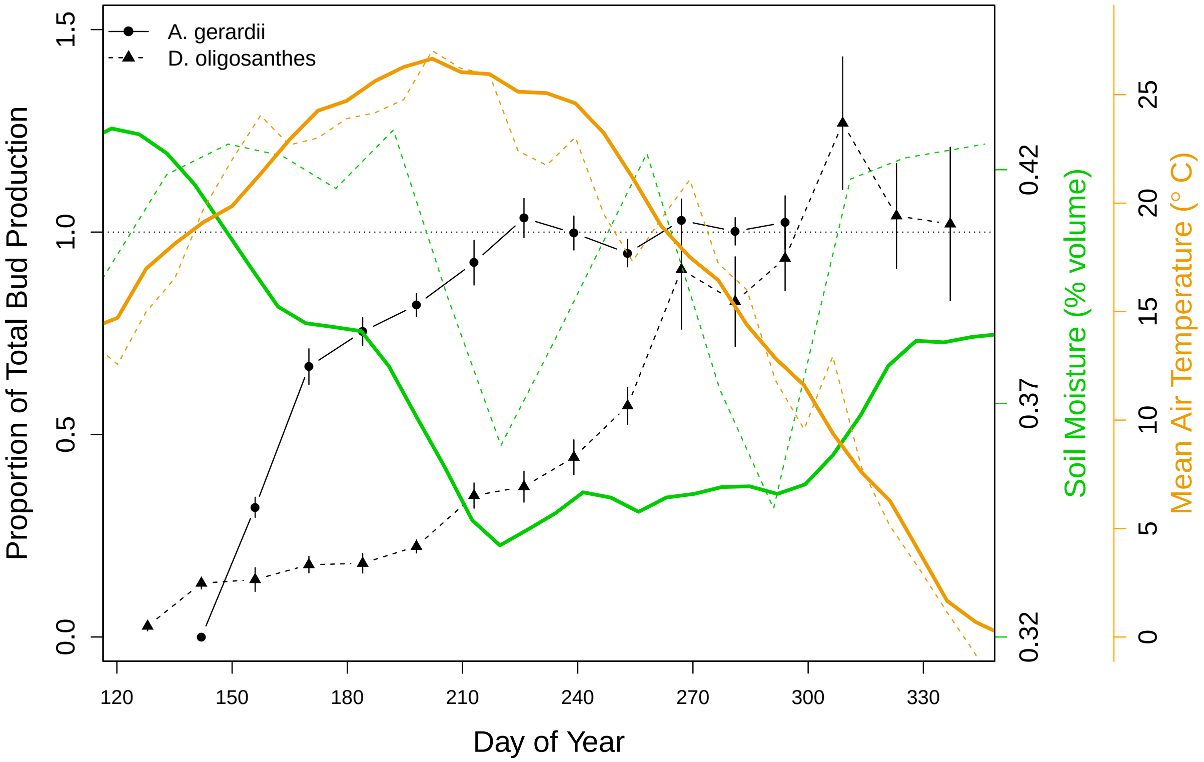

Figure 5.1: Bud production of *Andropogon gerardii* and *Dichanthelium oligosanthes* in 2008 as a proportion of total end-of-season buds per tiller. Thick solid lines are locally weighted polynomial regression (loess). The thick green line indicates the loess fit of daily soil moisture at 0.5 m from 1983-2011, pooled across years, fire regime, and grazing treatment at Konza Prairie Biological Station. The thick yellow line indicates the loess fit of daily mean air temperature from 1982 - 2011 Konza Prairie Biological Station. Thin dashed lines of the same colors are 2008 soil moisture and mean air temperature, respectively, for ungrazed watersheds that are burned every four years. Bud production data from Ott and Hartnett (2012) were redrawn with permission from J. Ott.

Table 5.1: Summary of the effects of drought and grazing on tallgrass prairie vegetation. Subscript numbers after column names indicate the chapter in which a disturbance was investigated. Arrows pointing up indicate that a response increased, arrows pointing down indicate that a response decreased, and (·) indicates no difference from the ambient control. Response that were not investigated in a chapter are indicated by "*na*."

Response	Group	Irrigation$_2$	Drought$_2$	Drought$_4$	Grazing$_3$	Grazing$_4$
Bud Density	Total	⇑	·	⇓	⇓	·
	Graminoid	⇑	·	·	⇓	·
	Forb	·	·	⇓	·	·
Stem Density	Total	⇑	·	⇓	⇑	·
	Graminoid	⇑	·	·	⇑	·
	Forb	·	·	·	·	·
Buds per Stem	Total	·	·	·	⇓	·
	Graminoid	·	·	·	⇓	⇓
	Forb	·	·	⇓	·	·
Stems per Bud	Total	·	·	*na*	⇑	*na*
	Graminoid	·	·	*na*	⇑	*na*
	Forb	·	·	*na*	·	*na*
ANPP	Total	·	⇓	⇓	*na*	⇑
	Graminoid	·	⇓	⇓	*na*	⇑
	Forb	·	·	⇓	*na*	⇑
Seed Bank Density	Total	*na*	*na*	*na*	⇑	*na*
	Graminoid	*na*	*na*	*na*	·	*na*
	Forb	*na*	*na*	*na*	⇑	*na*
Species Richness	Above	·	·	⇓	⇑	·
	Below	·	·	⇓	⇑	·
Shannon Diversity	Above	·	·	⇓	⇑	·
	Below	·	·	⇓	⇑	·
C_4 Grass Flowering	Total	·	⇓	⇓	*na*	·
Rhizome Biomass	Total	·	·	·	*na*	·