A DESERT IN DISGUISE: THE RESILIENCE OF THE NEBRASKA SANDHILLS

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A DESERT IN DISGUISE: THE RESILIENCE OF THE NEBRASKA SANDHILLS

by

Jeffrey C. Hartman

A DISSERTATION

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The Graduate College at the University of Nebraska
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A DESERT IN DISGUISE: THE RESILIENCE OF THE NEBRASKA SANDHILLS

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Adviser: David A. Wedin

The Nebraska Sandhills are the largest sand dune system in the Western Hemisphere, and are unique because they remain relatively undisturbed from row crop agriculture. Research in the past two decades demonstrated that the Sandhills are dynamic on millennial timescales, switching between stabilized, vegetated states to non-vegetated, mobilized states. The Sandhills are currently stabilized, but understanding how ecological processes are altered as sand dunes transition from stabilized to mobilized states, provides insight into the thresholds, stability, and resilience of this grassland ecosystem. My research investigated the impacts of vegetation disturbances on ecological processes and the sand dune surface stability. For ten years (2005-2014), Press, Pulse, Grazed, and Control treatments were applied to stabilized sand dunes. Press treatments killed vegetation and prevented re-growth, while Pulse treatments killed vegetation in one year and then allowed two years of recovery. I measured changes in key ecosystem processes (aboveground biomass, nitrogen cycling, soil respiration, and total soil C and N pools) as sand dunes transitioned from stable to mobile states. I found that Press vegetation disturbances surpassed the grassland’s resilience and led to mobile sand dunes after 4-5 years. However, there was a time lag between complete loss of aboveground vegetation and increased erosion, which indicated that belowground ecological functioning buffers disturbances and is important in controlling sand dune
surface stability. The Pulse treatment reduced aboveground biomass and shifted the system to an annual and C₃ grass dominated plant community, but belowground functioning continued at reduced rates and erosion was minimal. However, in the 11th year of the study, the spatial variance in erosion activity increased, potentially foreshadowing a state shift in the Pulse treatment. A severe drought in 2012 produced similar impacts on ecosystem processes as the Pulse treatment, reducing aboveground biomass and increasing the contribution of forbs. The 2012 drought also increased available soil nitrate and ammonium, reduced net N mineralization and soil CO₂ flux, but did not cause measurable erosion. My results indicate that the Nebraska Sandhills are resilient to climate and vegetation disturbances on decadal timescales, and that continued belowground functioning is critical to the stability of this ecosystem.
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CHAPTER 1 - INTRODUCTION

The Nebraska Sandhills are a complex ecosystem where land-cover, climate, management, and fluxes of water, carbon, nutrients, and energy have diverse ecological and economic impacts. The Sandhills are located in central and western Nebraska and are the largest sand dune system in the Western Hemisphere (>50,000 km²). This distinct ecoregion, situated between the tallgrass prairie in the east and the shortgrass prairie in the west, is unique in that it remains relatively undisturbed from row crop agriculture. The region makes up half of the 23 million acres of rangeland in Nebraska and is the foundation for Nebraska’s cattle industry which generates $7.2 billion each year (Nebraska Department of Agriculture 2013). The Nebraska Sandhills are situated over the northern portion of the High Plains Aquifer. The sandy permeable soil (85-96% sand) minimizes runoff and enhances infiltration, where an estimated 20% of mean annual precipitation is allocated to the groundwater (Billesbach and Arkebauer 2012), which recharges up to 30% of the groundwater in the High Plains aquifer (Bleed and Flowerday 1998).

Grassland Ecosystems & the Nebraska Sandhills

Globally, grasslands ecosystems are estimated to cover 31-43% (41-56 million km²) of terrestrial surface area (White et al. 2000). The majority of grasslands in North America occur in the Great Plains, which cover more than 3 million km² (Lauenroth et al. 1999), and extend from the Rocky Mountains in the west, to Illinois in the east, and from Texas and New Mexico in the south, to Canada in the north. Currently, much of the original expansive grassland range is gone, with on average 30% (range: 2.4% – 64.3%)
of the pre-European settlement grassland area remaining, as agriculture and urbanization have altered land-use and land-cover (Samson et al. 2004). Much of the remaining grasslands are utilized for cattle grazing. These regions provide a host of ecosystem services (wildlife habitat, C sequestration, grazing and haying land, groundwater recharge, etc.) and their productivity depends on the climate (precipitation/temperature), soil type, and disturbance regimes.

**Climate**

Great Plains grasslands are dominated by a continental climate, with a longitudinal (vertical) precipitation gradient moving from the western Great Plains (<300 mm yr\(^{-1}\)) to the eastern Great Plains (>1000 mm yr\(^{-1}\)). The temperature gradient across the Great Plains is perpendicular to the precipitation gradient, and increases from an annual mean of greater than 20 °C in the south, to less than 5 °C in the north (Epstein et al. 1996). Within the Great Plains spectrum, the Nebraska Sandhills are situated in the middle of both the temperature (9 °C) and precipitation (576 mm yr\(^{-1}\)) gradient that spans the Great Plains (Table 1-1). Generally, 75% of the precipitation falls during the growing season from April through September, with large variations (CV 10 – 40%) intra and inter-annually (Knapp and Smith 2001). Precipitation variation in the Great Plains grasslands occurs through altered total annual quantity, longer or shorter intervals between rainfall events, and changes in individual rain event sizes (Fay et al. 2008). Because of the variation in precipitation, drought is a common disturbance in Great Plains grasslands, reducing annual precipitation by 40% or more (Knapp et al. 2015). Within a growing season, the timing of drought is important (Craine et al. 2012a, Denton
2014), and it strongly influences primary productivity, both during the drought year and
the years after (Nippert et al. 2006, Thomey et al. 2011). Although droughts can be
severe and long-lasting, these ecosystems have co-evolved with these disturbance
regimes (Craine et al. 2012b), and display resistance and resilience to drought disturbance

**Fire & Herbivory**

Along with climate, fire and herbivory are generally the other main forms of
disturbance that affect ecosystem processes such as primary production, plant community
composition, and nutrient cycling. Fire and herbivory have a long-term association with
grasslands and are hypothesized as key contributors to the expansion of grasslands during
the Miocene (Anderson 2006). Herbivores can consume 15 – 60% of the ANPP, alter
plant community composition, affect plant growth structure, and contribute to nutrient
cycling (Collins et al. 1998, Anderson et al. 2006, Li and Guo 2014, VanderWeide and
Hartnett 2015). Fire occurs in most grasslands as a periodic disturbance (1-3 years) that
favors grass species over woody vegetation by removing dead vegetation and reducing
light competition for new shoots (Ratajczak et al. 2014). Fire also affects nutrient cycles
by releasing N during burns, with frequent fires reducing net N mineralization and
increasing plant demand for N (Knapp et al. 1998). In the Nebraska Sandhills, there is
historically little use of fire as it reduces productivity in choppy sand sites and there are
concerns it will increase the potential of sand dune blowouts (Pfeiffer and Steuter 1994,
Volesky and Connot 2000). The Sandhills region is largely utilized as grazing land for an
estimated half a million cattle (Nebraska Department of Agriculture 2013). Grazing in
the Sandhills is moderate, and ranges are generally stocked at 0.99 – 4.9 AUM ha⁻¹, depending on the range site, condition, and precipitation (Judy 2014). Because of this, grazing by cattle is the preferred management tool to alter grassland productivity, species composition, and forage quality (Mousel et al. 2011, Stephenson et al. 2013, Redden 2014).

**Primary Production**

Primary production in grasslands is strongly influenced by precipitation variability (Sala et al. 1988). However, temperature and soil texture also play a significant role in affecting primary productivity (Burke et al. 1998, Lane et al. 1998), illustrated by the variation in aboveground net primary productivity in the Great Plains (Table 1-1), from less than 100 g m⁻² in desert grasslands to greater than 450 g m⁻² in tallgrass prairies (Knapp and Smith 2001). ANPP in the Nebraska Sandhills varies from 100 – 300 g m⁻² (Table 1-1; Sridhar and Wedin 2009) depending on the topographic location. Across the Great Plains, the southern grasslands are dominated by warm-season C₄ grasses, with a shift to C₃ grass dominance in more northern locations (von Fischer et al. 2008). The Sandhills are situated between the three major central grassland types in the Great Plains (tallgrass, shortgrass, and mixed grass) so it contains a mixture of C₃ and C₄ grasses species from the surrounding regions (Barnes and Harrison 1982). This change in community composition across the Great Plains affects not only the productivity, but also vegetation quality (C:N) and nutrient cycling.
**Nutrient Cycling**

After water, nitrogen is generally the most limiting nutrient in grassland ecosystems, and low soil N availability is a common characteristic of temperate grasslands (Wedin 1995). There is a positive feedback between vegetation structure and N cycling (Wedin and Tilman 1992). Warm-season, C₄ grasses are strong competitors for soil N (Wedin and Tilman 1990) and have high N use efficiency (Gibson 2009), which is reflected in the low tissue N concentration (~1.34%; Tjoelker et al. 2005). Low quality litter decomposes slowly, which produce low mineralization rates and low N availability. This positive feedback system is almost completely regulated by biotic processes, and produces soil environments where warm season, C₄ grasses have a competitive advantage for soil N (Wedin 2004). The soil N availability in tallgrass prairies varies from 0.2 – 0.4 g N m⁻² with net N mineralization rates of 1 – 4 g N m⁻² during the growing season (Knapp et al. 1998) while shortgrass prairies generally have net N mineralization rates of 1.0 – 2.5 g N m⁻² yr⁻¹ and with a mean available N pool of 0.1 – 0.25 g N m⁻² (Lauenroth and Burke 2008). Arid grasslands contain a mean available N pool of 0.17 – 0.41 g N m⁻² (Hartley and Schlesinger 2000), while annual net N mineralization (estimated from plant uptake) can reach 5 g N m⁻² yr⁻¹ (Havstad et al. 2006). Heavy grazing affects the supply rate of N by returning a high proportion of the consumed N back to the system via urine (Augustine et al. 2013), and shifts in precipitation and drought can alter how effective plants and microbes are at acquiring N, increasing the potential for N losses from arid grasslands (Dijkstra et al. 2012).
Soil Texture

Soil texture varies across the grasslands in the Great Plains (Table 1-1) and affects water availability, decomposition, nutrient cycling, and primary productivity. Clay soils generally have higher field capacity of water compared to sandy soils, but under low annual precipitation, sandy soils actually have more plant available water (Noy-Meir 1973) as runoff is lower and water percolates much deeper (Sridhar et al. 2006a). Because of this, primary production is affected by soil texture (Burke et al. 1989), with a general increase in ANPP as the clay content of the soil increases (Epstein et al. 1997a). Recent studies have shown that soil type can also have large impacts on decomposition rates, with coarser textured soils (higher sand content) leading to increased decomposition rates (Epstein et al. 2002) and contributing more mineralized carbon to soil respiration (Jin and Goulden 2013). In the Nebraska Sandhills, the soils are more than 91% sand (Wang et al. 2008). Because of this, precipitation does not leave the system as runoff, but is stored as soil moisture, lost through evapotranspiration, or recharged to the High Plains Aquifer (Chen and Chen 2004, Szilagyi et al. 2011, Billesbach and Arkebauer 2012). The sandy soils also contribute to soil water stress, where even fully saturated soils can be depleted past wilting point in a just a few weeks during the summer, when potential evapotranspiration is high.

Carbon Sequestration

Soils are the largest pool of C in terrestrial ecosystems, and on a global scale, soils hold 1500 Pg C in the top 1m of soil (Amundson 2003) in the form of soil organic matter (SOM). This soil organic matter is a major component of grassland
biogeochemical cycles and the amount and quality of SOM can affect ecosystem processes (Schmidt et al. 2011). Grasslands in the Great Plains vary in the organic matter content, ranging from 0.1% in southern desert grasslands to 2 – 5% OM in more northern tallgrass prairies (Table 1-1). The organic matter content of the Nebraska Sandhills varies as well, with 0.7 – 0.8% organic matter on sand dune slope and ridge locations, and higher organic matter content (1.5% OM) in swale locations between dunes (Schacht et al. 2000). The ability of the Great Plains grasslands to sequester C is affected by the balance of plant inputs and decomposition, which is affected by precipitation, temperature, and soil texture. There is potential to manage for C sequestration in Great Plains grasslands, but the combined effects of increasing atmospheric CO₂ concentration, temperature, and altered precipitation regimes lead to uncertain predictions on the ability of the grassland soils to sequester carbon (Jones and Donnelly 2004, Mengistu and Mekuriaw 2014). Additionally, increasing N deposition does increase C sequestration in grassland soils (Fornara and Tilman 2012), but potential changes in community composition may obscure this effect.

**Ecological History of Sandhills**

Like most temperate grasslands, the Nebraska Sandhills are strongly influenced by fire frequency, grazing pressure, and climatic variations. Currently, the Nebraska Sandhills are stabilized by a relatively thin cover of mixed-grass prairie vegetation, but research over the last two decades demonstrated that the Sandhills have lost their stabilizing plant cover and migrated freely as wind-blown sand dunes several times during the last 10,000 years (Goble et al. 2004, Schmeisser McKean et al. 2014).
Originally, the Sandhills were viewed as a remnant from the Pleistocene (Smith 1965), but radiocarbon dating of organic rich soils within the sand dunes and optically stimulated luminescence (OSL) dating of quartz grains indicated that the sand dune formations were much younger, and activity in the region occurred several times during both the Pleistocene and Holocene (Mason et al. 2004, Goble et al. 2004).

OSL dating techniques provide a powerful tool to date the age of the sand dune compared to radiocarbon dating, which relies on organic material that is relatively sparse in the Sandhills compared to other grassland ecosystems. OSL dating of quartz grains gives a determination of the time since the grains were last exposed to sunlight during aeolian transport (Stokes and Swinehart 1997). Both radiocarbon and OSL dating have been combined to construct a history of dune mobilization and stabilization during the Pleistocene and Holocene (Miao et al. 2007b). It is estimated that this cycle has occurred several times in the last 10,000 years, varying between rapid changes in dune turnover (100 – 200 years) and longer periods (500+ years) of dune activity (Miao et al. 2007a, Mason et al. 2011). Dune activity within the Sandhills is spatially variable (Forman et al. 2001, Schmeisser McKean et al. 2014), but the best estimates indicate several widespread episodes of dune activity within the Sandhills that varied in duration and intensity (Figure 1-1; Loope and Swinehart 2000).

Although the interval and intensity of dune activity varies, there is evidence that it is linked with changes from a wet to a dry climate over the region (Mason et al. 2004, Nicholson and Swinehart 2005). Periods of hydrological drought correspond with increased dune activity, and at least three mega-droughts (droughts extending over a
period of decades or longer) have occurred within the Holocene which caused high rates of dune activity across large portions of the Sandhills (Hassan et al. 1997, Mason et al. 2004, Schmieder et al. 2011). The most recent period of aeolian activity occurred 800-1000 years before present, during the period known as the Medieval Climatic Anomaly (Cook et al. 2004, Sridhar et al. 2006b, Schmeisser et al. 2009). These droughts and subsequent dune activity are hypothesized to be caused by large wind shifts over the region (Sridhar et al. 2006b, Schmeisser et al. 2010). Generally, most of the moisture for the region is transported by the spring and summer winds from the south as they move moist air from the Gulf of Mexico over the Great Plains. Evidence from the last major period of dune activity (during the Medieval Climatic Anomaly) indicates that there was a large scale shift in wind patterns to a dry, southwesterly flow during the spring and summer (Sridhar et al. 2006b) that led to a drier climate and widespread dune activity.

The combined evidence from carbon dating, lakes levels through diatom analysis, and OSL dating (Schmieder et al. 2011) shows that the Nebraska Sandhills have undergone several episodes of dune mobilization and stabilization. These cycles of ecosystem collapse, and subsequent recovery, demonstrate that the Nebraska Sandhills are dynamic on millennial timescales, and is the reason why the region has been dubbed “a desert in disguise” (Sletto 1997, Loope and Swinehart 2000).

**Ecological Resilience**

The Nebraska Sandhills have clear alternative stable states, moving from stable, grass-covered dunes to mobile, blowing sand dunes. These clear alternative stable states make the Sandhills a good model system to apply the concepts of resilience theory
Resilience theory was originally introduced to help understand the capacity of ecosystem to withstand perturbations and remain within the original state (Holling 1973). Additionally, resilience theory helps explain how ecosystems with alternative states may be brought to a threshold by perturbations, undergo a critical transition, and emerge in a different stable state with new drivers and processes (Gunderson and Holling 2002). The ecological resilience of an ecosystem is defined as the ability to absorb a disturbance, reorganize, and still retain the same identity as before (Folke et al. 2010). Highly resilient systems are able to withstand perturbations and remain in the same stable state, with the same identity. The less resilient the system is, the more likely a perturbation is to push the system across a threshold into an alternative stable state. Additionally, multiple perturbations can potentially erode the ecological resilience of a system, as well as changes in external conditions (Folke et al. 2002).

These concepts can be applied to the Nebraska Sandhills to determine the resilience of this sand dune grassland ecosystem. Past research demonstrated that the Nebraska Sandhills have gone through several cycles of dune mobilization and stabilization (Loope and Swinehart 2000, Sridhar et al. 2006b, Mason et al. 2011), and that these cycles correspond with mega-drought events and shifts to a drier climate (Schmeisser et al. 2010, Schmieder et al. 2011). This evidence suggests that the Sandhills have low resilience to severe, long-term (centuries) drought, and are likely to undergo transitions in the future (Schmeisser et al. 2009). However, even recent severe droughts (perturbations) during the 1930’s and 1950’s were not sufficient to destabilize
the sand dune ecosystem (Weaver and Albertson 1936, 1939). These recent droughts suggest that although community composition and relative abundance of C\textsubscript{4} grasses changed, the grassland ecosystem surprisingly lost little grass cover and retained structure, function, and feedbacks (Weaver 1944). One hypothesis for Sandhills stability during the severe droughts of the last century is that they were relatively short, and the drought conditions did not occur through a westward shift in winds over the region as it did during the Medieval Climatic Anomaly (Sridhar et al. 2006b). While these mega-droughts and mobilization events have occurred in the past, and are likely to occur in the future, the disturbances required to initiate dune mobilization, and the thresholds for these events, are not well understood (Schmeisser et al. 2009, 2010). Although dune mobilization and stabilization is a geologic process, it is also an ecological one. Dune mobilization and stabilization is a cycle of ecosystem collapse and recovery, and above and belowground ecosystem processes control sand dune stability as the system moves from one state to another.

**Purpose**

The Nebraska Sandhills are a unique grassland ecosystem, composed almost entirely of sandy soils (>90%), straddling the mesic tallgrass prairie to the east and the arid shortgrass prairie to the west. Climate, fire, and grazing are major factors structuring the patterns and processes in the Sandhills. The droughts in the 1930’s and 1950’s demonstrated the resilience of the Sandhills, but what level of disturbance is required to initiate a transition from a stable to a mobile state? When this occurs, how long does the transition take and how are ecosystem processes altered during that transition? Does the
resilience of the Sandhills change in response to persistent, periodic disturbances? How important is the aboveground vegetation relative to the belowground root-soil structure in buffering against wind erosion? Additionally, responses to drought, grazing, fire, warming, and elevated CO₂ are well documented in many temperate grasslands (Parton et al. 2007, von Fischer et al. 2008, Carol Adair et al. 2011, Augustine et al. 2014, Burri et al. 2014), but there is relatively little research on similarities or differences of grassland ecosystem functioning in the Nebraska Sandhills. Because of this, some major questions include: How do the Sandhills fit into the spectrum of temperate grasslands in the Great Plains? How does climate variability (inter and intra annual variation) influence ecosystems processes? How are these components affected by grazing? How resilient are the Sandhills to perturbations, and how are the responses to climate perturbations different or similar to experimental vegetation disturbances? My research investigated the impacts of disturbances (vegetation, climate, grazing) on the Sandhills grassland ecosystem. I used empirical measurements to determine changes in key ecosystems processes (aboveground biomass, nitrogen cycling, soil respiration, and total soil carbon and nitrogen pools) inter-annually, intra-annually, and as sand dunes transitioned from stable and grass covered, to wind-blown mobile sand dunes.

**Study Site**

I conducted my research at UNL’s Barta Brothers Ranch (BBR) in the eastern Sand hills (Figure 1-2; Wang et al. 2008, Sridhar and Wedin 2009). The BBR (42°14’N, 99°39’W; elevation = 765 m) was established in 1996 with land donated to the University of Nebraska Foundation from Jim and Clifford Barta. BBR is about 2,200 ha in size with
2,000 ha categorized as upland range. The ranch is operated by UNL’s Northeast Research and Extension Center and serves as a model ranch that provides the facilities and support needed to conduct research and demonstrations relevant to Sandhills ranchers.

BBR experiences a typical mid-continental climate located within the transition zone between semiarid and sub-humid climates (Stephenson et al. 2013) and is characterized by periodic droughts and large seasonal and inter-annual variability in rainfall. From 1961 – 2000, the mean (±1 SE) annual precipitation was 576 ± 20 mm (High Plains Regional Climate Center 2015), with 50% falling in May, June, and July (Bleed and Flowerday 1998) and 76% of the precipitation occurring during the growing season from April to September. During the course of the study from 2004-2014, mean annual precipitation was 540 ± 36 mm, with above average wet years from 2008-2011 and an extreme drought in 2012 (Figure 1-3), where total annual precipitation was 45% (257 mm) of the long-term average. Mean monthly air temperatures range from -4.56 ± 0.91 °C in January to 23.37 ± 0.56 °C in July. The mean annual air temperature from 1961-2000 was 8.1 ± 0.3 °C (High Plains Regional Climate Center 2015), but during the course of this study from 2004-2014, the mean annual air temperature was 8.9 ± 0.3 °C, with eight of the eleven years higher than the 1961-2000 average (Figure 1-3). Similarly, the number of growing degree days (GDD) from 1961-2000 averaged 631 GDD, but from 2004-2014 the average increased to 717 GDD, with eight of the eleven years above the long-term average (Figure 1-3). During the drought of 2012, the daily high air temperatures reached a mean of 36.3 ± 0.6 °C in July, and mean annual air temperature
for the year (10.7 °C) was 1.8 °C and 2.5 °C higher than the 2004-2014 average and the 1961-2000 average, respectively (Figure 1-3). Similarly, the accumulated growing degree days in 2012 reached 943 GDD, 30% and 50% higher than the 2004-2014 and 1961-2000 average.

The landscape at BBR contains about 10% (200 ha) of contiguous subirrigated meadow, discontinuous subirrigated meadows, and wetlands. The other 90% of the ranch (2,000 ha) is categorized as upland mixed-grass prairie (Schacht et al. 2000). The sand dune soils are mostly composed of Valentine fine sand soils with an average of 94% sand and 0.61% OM at 30 cm depth (Soil Survey Staff et al. 2013). The sand dune areas are oriented west by northwest to east by southeast (5 – 15% slope, total relief <20m; Sridhar and Wedin 2009) and are covered by a mixed-grass prairie assemblage. Interdunal swale locations have greater soil carbon content (0.51%) compared to dune slopes and ridges (0.33%) in the top 30cm, with slightly less sand in the top 10cm of swale locations (91.2% sand) compared to dune slopes and ridges (94.4% sand). The A horizons of the swales extend to an average depth of 22.8cm (range 6-41cm), while A horizons on the dune ridges extend to an average depth of 11.8cm (range: 6-21cm; Wang et al. 2008). This upland mixed-grass prairie community is composed of a mixture of warm and cool season grasses, sedges, forbs, and shrubs. The aboveground net primary production of this mixed-grass community ranges from 100 – 300 g m$^{-2}$ (Wang 2008). Topographic position and aspect across a dune profile affect community composition, with interdunal swale areas (15% of landscape) having a higher proportion of switchgrass (Panicum virgatum), bluegrass (Poa pratensis), blue grama (Bouteloua gracilis), and sedges (Carex
eleocharis). The dune slopes and ridges (85% of landscape) are composed of warm-season, C₄ grasses such as prairie sandreed (Calamovilfa longifolia) and sand bluestem (Andropogon hallii), which are generally located on south facing slopes, while the north slopes contain more cool-season, C₃ grasses such as needle and thread grass (Hesperostipa comata), prairie june grass (Koeleria macrantha), and the shrub leadplant (Amorpha canescens) (Schacht et al. 2000, Milby 2011).

**Experimental Design**

My research continued a portion of the Grassland Destabilization Experiment (GDEX), a landscape-scale experiment examining the role of vegetation in dune stability. The GDEX project was part of the Sandhills Bio-complexity Project, funded by NSF from 2003-2008. The goal of the GDEX project was to determine how fluxes of energy, water, and nutrients change as the land cover changes from continuous grass cover to bare sand. The GDEX project was started in 2004 and was set up with ten circular plots, 120m in diameter (1.1 hectares total area), which span the profile of one sand dune, from swale to dune top to swale (Figure 1-2). Within each plot, there were 22 subplots, with locations covering the sand dune profile, varying in elevation, aspect, and slope. Measurements on aboveground biomass, soil respiration, soil N availability, and total soil carbon and nitrogen were collected at the subplot scale.

At the beginning of the GDEX project in 2004, cattle exclosures were constructed around eight of the plots (Plots 1-8), with plots 9 and 10 (Grazed treatment) remaining in the deferred rotational grazing (DRG) studies conducted at Barta Brothers Ranch, where the grazing is moderate and well managed (Schacht et al. 2010, 2011). Two plots were
assigned to one of five treatments (Figure 1-2, Table 1-2), which spanned the range of continuous grass cover to bare sand. Treatments included a Control, a Grazed treatment, a Pulse treatment, a Press treatment, and an Aggressive treatment. Press and Pulse treatments were chosen for the GDEX project to provide insights into the stability and resilience of the sand dune grasslands (Bengtsson et al. 2003, Collins et al. 2011). Permanent and sustained Press vegetation disturbances demonstrated the time frame required to overcome the resilience of the ecosystem, while short-term Pulse vegetation disturbances (pulse-recovery) showed the ability of the Sandhills grassland ecosystem to recover from periodic, cyclical disturbances. Vegetation in the Press and Aggressive treatments was killed and kept dead with herbicide applications, while vegetation in the Pulse treatment was subjected to three-year pulse-recovery cycles, with vegetation killed with herbicide in one year, and then allowed two years of recovery.

The Control plots (2 and 4), beginning in May 2004 were excluded from grazing, and were not subject to any experimental vegetation or physical soil disturbances (Figure 1-2, Table 1-2). The Grazed treatment plots (9 and 10) were located in pastures that were included in the Barta Brothers DRG studies (Schacht et al. 2010, 2011, Stephenson et al. 2013, 2015). The two Grazed treatment plots (9 and 10) were located in the southeast corner of the N5 pasture (61.9 ha) and the southwest corner of the N6 pasture (57.1 ha) (Figure 1-2, Table 1-2). Pastures in the DRG study were stocked with cow-calf pairs (1.5 Animal Unit Equivalent) during the grazing season, from May 15 to October 15. From 2004-2008, pastures were stocked with 127-135 cow-calf pairs (1.5 AUE), at a stocking rate of 1.65-2.12 Animal Unit Months (AUM) ha\(^{-1}\), and grazed over three periods (2-14
days in length) in May, June, and August. From 2009-2014, pastures were stocked with 60-62 cow-calf pairs (1.5 AUE), at a stocking rate of 1.70-2.09 AUM ha\(^{-1}\). Pastures were grazed over a single period (30-42 days in length), rotating between May-June, June, July, August-September, and September-October grazing periods. Because the Grazed treatment plots were located in different pastures (Figure 1-2), they were never grazed at the same time in the deferred rotational grazing studies, but received similar grazing impacts over the course of the ten-year GDEX study.

In the Pulse, Press, and Aggressive treatments, killing the grass and broadleaf vegetation was done by using applications of Roundup Weathermax (glyphosate 49%), combined with Amine 2,4-D (47%). The initial burn down of the grassland vegetation in the Aggressive, Press, and Pulse treatments was done using by using a tank mixed combination of glyphosate and 2,4-D, applied at a rate of 5.12 L ha\(^{-1}\) of glyphosate and 5.12 L ha\(^{-1}\) 2,4-D. Subsequent applications of herbicide to suppress vegetation re-growth was applied at lower rates, because the vegetation height and density was reduced compared to the pre-treatment grassland vegetation state. The herbicide reapplications were applied by using a tank mixed combination of glyphosate and 2,4-D, applied at a rate of 1.68 L ha\(^{-1}\) of glyphosate and 1.68 L ha\(^{-1}\) 2,4-D. Herbicide was applied by using an ATV mounted 25-gallon boom sprayer.

The Press treatment started on 5/19/2005, with an initial herbicide application at the higher rate (5.12 L ha\(^{-1}\)) to kill the grassland vegetation in plots 1 and 8 (Figure 1-2). Vegetation re-growth was prevented with 15 further herbicide applications (reduced rates 1.68 L ha\(^{-1}\)), on 5/27/2005, 7/29/2005, 8/11/2005, 5/6/2006, 7/26/2006, 4/8/2007,
5/4/2007, 5/16/2007, 6/13/2007, 5/15/2008, 7/3/2008, 5/1/2009, 6/17/2009, 6/4/2010, and 7/3/2010. No physical disturbances, other than herbicide application, occurred in this treatment. The Press treatment was discontinued at the end of 2010 because the plots were fully mobilized sand dunes, and efforts began in 2011 to begin stabilization and re-vegetation. The Press treatment plots were assigned to the new Dune Re-vegetation Experiment (DREX), where the goal was to determine the effects of grass, tree, and shrub species on the stability, productivity, and hydrology of the Sandhills. Data from the former Press treatment plots used in the new DREX study (2011-2014) are not used in my analyses, as it was not the focus of my research.

The Pulse treatment is a 3-year cycle, with vegetation killed and re-growth prevented in first year, and then in the second and third year the vegetation is allowed to recover (Table 1-2). To date, there have been three full cycles, with herbicide applications in 2005, 2008, 2011 and two years of recovery between each Pulse herbicide treatment year. A fourth treatment cycle started in 2014, with 2015 and 2016 planned as recovery years. The Pulse treatment started on 5/19/2005, with an initial application of herbicide at the higher rate (5.12 L ha\(^{-1}\)) to kill the grassland vegetation in plots 3 and 6 (Figure 1-2). Vegetation re-growth in 2005 was prevented with a further herbicide application at the reduced rate (1.68 L ha\(^{-1}\)) on 5/27/2005, 7/29/2005, and 8/11/2005. Vegetation in this treatment was then allowed to recover in 2006 and 2007. A second Pulse treatment cycle started in 2008, with herbicide applications (1.68 L ha\(^{-1}\)) on 5/15/2008 and 7/3/2008. Vegetation was then allowed to recover in 2009 and 2010. A third Pulse treatment cycle started in 2011, with a single herbicide application (1.68 L ha\(^{-1}\)
Vegetation was allowed to recover in 2012 and 2013. A fourth Pulse treatment cycle started in 2014, with herbicide applied (1.68 L ha\(^{-1}\)) on 6/5/2014, and vegetation allowed to recover 2015 and 2016.

The Aggressive treatment (plots 5 and 7) is similar to the Press treatment (Figure 1-2, Table 1-2). Grassland vegetation in the Aggressive treatment was killed on 9/9/2004 with the higher herbicide application rate (5.12 L ha\(^{-1}\)). However, physical disruption of the soil surface was included in this treatment, where the dead vegetation was raked with an ATV, followed by a shallow disking of the soil surface. Further applications of herbicide to prevent vegetation re-growth (at the reduced rate of 1.68 L ha\(^{-1}\)) were applied on 9/17/2004, 10/6/2004, 6/16/2005, 5/6/2006, 7/27/2006, 5/16/2007, 6/13/2007, 5/15/2008, 7/3/2008, 5/1/2009, and 6/17/2009. The Aggressive treatment plots were fully mobilized after two years, and were discontinued at the end of 2010. Like the Press treatment plots, the Aggressive treatment plots were included in the new DREX study at the beginning of 2011. Data from the Aggressive treatment is not used in my analyses, as it was not the focus of my research.

My dissertation used data from past measurements of the GDEX treatments (2004 – 2011), and I continued the remaining treatments (Control, Grazed, and Pulse) from 2012 – 2014 (Sridhar & Wedin 2009; Istanbulluoglu, Wang & Wedin 2012). The following chapters will focus on aboveground (vegetation) and belowground (N availability, soil respiration, total soil C and N) ecological processes, and how these aspects of ecological functioning confer stability and resilience to the grassland sand dune ecosystem of the Nebraska Sandhills.
Figures & Tables

Table 1-1. Characteristics for five LTER grassland sites and for the UNL owned Barta Brothers Ranch (BBR) in the central Sandhills. LTER site data was gathered from the Long Term Ecological Research Network (LTER 2013). Data for the Sandhills site (BBR) was obtained from Wang et al. (2008) and Sridhar and Wedin (2009).

<table>
<thead>
<tr>
<th>Site</th>
<th>Type</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>OM (%)</th>
<th>MAT (°C)</th>
<th>MAP (mm)</th>
<th>ANPP (g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedar Creek</td>
<td>Savanna / Tallgrass</td>
<td>90</td>
<td>5</td>
<td>5</td>
<td>2.4</td>
<td>6.7</td>
<td>801</td>
<td>277.3</td>
</tr>
<tr>
<td>Shortgrass Steppe</td>
<td>Shortgrass Steppe</td>
<td>70</td>
<td>20</td>
<td>10</td>
<td>0.2-0.6</td>
<td>8.6</td>
<td>320</td>
<td>116.5</td>
</tr>
<tr>
<td>Sandhills</td>
<td>Mixed Grass</td>
<td>94</td>
<td>5</td>
<td>1</td>
<td>0.7-1.5</td>
<td>9.29</td>
<td>576</td>
<td>189.5</td>
</tr>
<tr>
<td>Konza</td>
<td>Tallgrass</td>
<td>5</td>
<td>60</td>
<td>35</td>
<td>2.3-5.0</td>
<td>12.9</td>
<td>835</td>
<td>442.6</td>
</tr>
<tr>
<td>Sevilleta</td>
<td>Desert Grassland</td>
<td>70</td>
<td>20</td>
<td>10</td>
<td>0.1-0.3</td>
<td>13.4</td>
<td>244</td>
<td>55.5</td>
</tr>
<tr>
<td>Jornada</td>
<td>Desert Grassland</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>0.1-0.3</td>
<td>17.2</td>
<td>298</td>
<td>229.5</td>
</tr>
</tbody>
</table>
Table 1-2. Description of the GDEX treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Years Active</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong> (Plots 2 &amp; 4)</td>
<td>2004-2014</td>
<td>Un-grazed&lt;br&gt;No herbicide&lt;br&gt;No physical disturbance</td>
</tr>
<tr>
<td><strong>Grazed</strong> (Plots 9 &amp; 10)</td>
<td>2004-2014</td>
<td>Moderate, deferred rotational grazing</td>
</tr>
<tr>
<td><strong>Pulse</strong> (Plots 3 &amp; 6)</td>
<td>2005-2014</td>
<td>3 year cycle: 1 year of vegetation death, 2 years of recovery&lt;br&gt;Plots killed with herbicide in 2005, 2008, 2011, and 2014&lt;br&gt;No physical disturbance or grazing</td>
</tr>
<tr>
<td><strong>Press</strong> (Plots 1 &amp; 8)</td>
<td>2005-2010</td>
<td>Vegetation killed and re-growth prevented with herbicide&lt;br&gt;Permanent vegetation death beginning in May 2005&lt;br&gt;No physical disturbance or grazing</td>
</tr>
<tr>
<td><strong>Aggressive</strong> (Plots 5 &amp; 7)</td>
<td>2004-2010</td>
<td>Vegetation killed and re-growth prevented with herbicide&lt;br&gt;Permanent vegetation death beginning in Sept 2004&lt;br&gt;Vegetation raked and soil disturbed with shallow disking</td>
</tr>
</tbody>
</table>
Figure 1-1. Estimates of intervals and relative intensities of sand dune activity in the Nebraska Sandhills over the last 10,000 years. Figure modified from Nicholson and Swinehart (2005).
Figure 1-2. A) Outline of the Nebraska Sandhills with the location of the Barta Brothers Ranch (BBR) and B) a grayscale Google Earth image (September 20th, 2014) of the GDEX project site a decade after the start of the project. Each plot (10 total; 120m diameter) is circled and labeled with the plot number. Two plots were assigned to each treatment in 2004. Plots 1-8 were fenced within the grazed pastures (N5-N8) to exclude cattle.
Figure 1-3. Climate data (2004-2014) from a High Plains Regional Climate Center (HPRCC) Automated Weather Data Network (AWDN) station located near the GDEX site at Barta Brother’s Ranch in the Nebraska Sandhills. Panels are: A) monthly precipitation (mm), B) cumulative precipitation annually (mm), C) accumulated growing degree days annually, and D) mean (±1 SE) annual temperature (°C). In panels B, C, and D, solid black lines represent the long-term average (1961-2000), and dashed red lines represent the 2004-2014 average.
CHAPTER 2 - EXPERIMENTAL VEGETATION DISTURBANCES ALTER PLANT COMMUNITY COMPOSITION AND ABOVEGROUND BIOMASS IN THE NEBRASKA SANDHILLS

Abstract

Aboveground net primary production (ANPP) is a key ecosystem characteristic, which integrates fluxes of matter and energy, and is useful metric to compare productivity across terrestrial ecosystems. In the Nebraska Sandhills, ANPP provides the economic basis for cattle ranching in the region and serves to stabilize the sandy soils from erosion. Historically, this region has undergone widespread dune activity several times in the last 10,000 years, so it is important to understand the buffering capacity of aboveground vegetation to erosion, as well as determining how grasslands respond to altered disturbance regimes. The purpose of this research was to determine how experimental vegetation disturbances (Press and Pulse), climate variability (intra and inter annual), and topographic position across the landscape contribute to the variation in aboveground live biomass (ANPP estimate) and the persistence of aboveground biomass. Aboveground biomass was sampled monthly during the growing season (April-October) from 2005-2014, by hand clipping 0.25 m² quadrat frames at a designated dune ridge and swale location. Aboveground biomass was then sorted into forb, grass, shrub, and dead fractions, dried, and then weighed. I found the aboveground biomass in the Nebraska Sandhills to be resilient to drought, grazing, and experimental vegetation disturbances. Peak aboveground live biomass, weighted by landscape topography, averaged 160.1 g m⁻²
on un-grazed dunes, and I estimated a $C_3/C_4$ ratio of 55:45. In general, the grass component (71.9%) dominated peak aboveground live biomass on un-grazed dunes, with interdunal swales producing more than two times the total live biomass as dune ridges. In both Control and Grazed treatments, peak aboveground biomass production occurred in July. I estimated the residence time of aboveground biomass to be 1.9 – 2.5, depending on topographic position and grazing. A severe drought in 2012 reduced aboveground live biomass by 26 – 54%, but productivity recovered to average levels in one to two years. Experimental Pulse disturbances reduced aboveground biomass and shifted community composition to an annual and $C_3$ dominated plant community (~96% $C_3$), and the peak aboveground biomass production occurred later in the growing season in September. After three cycles of Pulse disturbances, the sand dunes were still intact, although observational evidence suggested a threshold is approaching. My results demonstrate the resilience of the Nebraska Sandhills to both drought and experimental vegetation disturbances on a decadal timescale. However, Press disturbances managed to fully mobilize these sand dunes in 4 – 5 years by preventing vegetation growth, while chronic pulse disturbances do have the ability to alter the plant community structure, function, which ultimately may shift the sand dune grasslands into a mobilized state.

**Introduction**

Aboveground net primary production (ANPP) is a key ecosystem characteristic, which integrates matter and energy fluxes (Ruppert and Linstädter 2014), and is useful metric to compare productivity across terrestrial ecosystems (Knapp and Smith 2001). Variation in ANPP of temperate grasslands is constrained by a host of factors such as
intra- and inter-annual climate variability (Epstein et al. 1997b, Hsu et al. 2014), grazing management (Beck et al. 2014, Borer et al. 2014), community composition (Isbell et al. 2013), fire (Ratajczak et al. 2014), and nutrient availability (Avolio et al. 2014). Measuring these fluctuations is important because ANPP influences virtually all ecosystem processes, such as decomposition (Hobbie 2015), N mineralization (Reich et al. 2001), soil respiration (Fay et al. 2011), and carbon storage (Derner et al. 2006). Understanding the variability in ANPP, and the cascading effects on ecosystem processes, is important to predict potential responses of temperate grasslands to future management and climate change impacts (Polley et al. 2014).

Although temperate grasslands in the Great Plains evolved under climate variability and grazing pressures, many ecosystem characteristics and processes are highly responsive to these disturbance regimes. In numerous studies, drought and experimentally reduced precipitation decreased ANPP by up to 60%, with associated decreases in plant cover and disproportionate effects on shallow rooted species (Craine et al. 2011, Cherwin and Knapp 2012, Hoover et al. 2014c, Orsenigo et al. 2014). Within a growing season, the timing of drought is important (Craine et al. 2012a, Denton 2014), and it strongly influences primary productivity, both during the drought year and the years after (Nippert et al. 2006, Thomey et al. 2011). Increased temperatures and climate extremes may increase productivity in spring months and favor C3 species (Sherry et al. 2008), but higher temperatures in the late season can severely reduce productivity (Sherry et al. 2008, Fay et al. 2011, Hoover et al. 2014b). Grazing impacts both above and belowground processes, altering root to shoot ratios and belowground production (López-
Márscico et al. 2015), carbon storage (Derner et al. 2006), and nutrient cycling (Singer and Schoenecker 2003). Grazing can remove >50% of ANPP (Gibson 2009) and can increase species diversity, depending on the availability of resources and length of grazing history (Cingolani et al. 2005, Collins and Calabrese 2012). The spatial and temporal patterns of grazer activity also can greatly alter the effects of other disturbances, such as increasing the potential for soil erosion, removing fuel for fire, or altering plant responses to drought (Heitschmidt et al. 2005, Blair et al. 2014). In susceptible grasslands, poorly managed grazing can reduce the resilience of the ecosystem, leading to a transition from one ecological state, to another, less desirable state (Briske et al. 2005).

Disturbances such as droughts in the Great Plains can be severe and long-lasting, but these ecosystems have co-evolved with these disturbance regimes (Craine et al. 2012b), and display resistance and resilience to drought disturbance events (Vogel et al. 2012, Hoover et al. 2014a). The infamous Dust Bowl droughts of the 1930’s reduced grassland cover by 50 – 95% (Weaver et al. 1935, Weaver and Albertson 1936), yet the system displayed slow recovery by 1944 – 45, although with major shifts in plant community composition (Weaver 1943, Weaver and Albertson 1943, Albertson and Weaver 1944, 1946). Although semi-permanent drought conditions are not likely for the Great Plains (Hoerling et al. 2012), altered precipitation and increased temperatures are likely (IPCC 2013). Determining how grasslands respond to altered disturbance regimes is important to predict responses to these projected climate change impacts.

Within the Great Plains, the Nebraska Sandhills are a keystone ecosystem, occupying over 50,000 km², situated between the tallgrass prairie to the east and the
shortgrass steppe to the west. Currently, this sand dune grassland (> 90 % sand) is stabilized by a relatively thin (70% of roots in top 20cm; ANPP: 100-300 g m⁻²) cover of mixed-grass prairie (Wang et al. 2008). Although this temperate grassland has co-evolved with grazing pressures and climate variability, recent research has determined that these grass-stabilized dunes displayed major spatial and temporal dune activity several times in the last 10,000 years (Loope and Swinehart 2000, Miao et al. 2007b), with the most recent occurrence during the MCA 700-1,000 years ago (Mason et al. 2004). Outside of the mega-droughts hypothesized to initiate dune activity (Sridhar et al. 2006b), aboveground live and dead vegetation are assumed to have a protective role against wind erosion (Li et al. 2005), and any loss of plant cover potentially leads to blowouts (Stubbendieck et al. 1989). However, the relative ability of aboveground live and dead vegetation to buffer wind erosion is emphasized in shrub-dominated sand dunes (Mora and Lázaro 2013, Bhattachan et al. 2014), but in grassland dune systems, the structural and functional buffering capacity of the belowground root-soil complex may be as important. Relating loss of aboveground vegetation to erosion, and estimating residence times of aboveground biomass will provide insights into the relative ability of aboveground vegetation to buffer erosion.

My research used aboveground biomass data from the Grassland Destabilization Experiment (GDEX), a decade-long project (2005-2014) in the eastern Nebraska Sandhills which investigated the effects of grassland destabilization on energy, water, and nutrient fluxes (Wang et al. 2008, Sridhar and Wedin 2009, Istanbulluoglu et al. 2012). The GDEX project used Control and Grazed treatments, along with experimental Pulse
and Press vegetation disturbances, to provide insights into the stability and resilience of the sand dune grasslands (Bengtsson et al. 2003, Collins et al. 2011). The Press treatment permanently kills vegetation, while the Pulse treatment kills vegetation for one year and then allowed two years of recovery. The purpose of this research was to determine how experimental vegetation disturbances, grazing, climate variability (intra and inter annual), and topographic position across the landscape contribute to the variation and persistence in aboveground live biomass. I expected topography (soil moisture) and intra and inter annual differences in precipitation to drive the variation in aboveground live biomass, and the relative percentage of forb and grass fractions. I predicted grazing would reduce live forb, grass, and total biomass across topographic locations and reduce the residence time \( RT = \frac{\text{total aboveground biomass}}{\text{live aboveground biomass}} \) of aboveground biomass. I hypothesized that the Pulse treatment would reduce annual peak aboveground live biomass (an estimate of ANPP), and increase the relative contribution of forbs to total aboveground live biomass. Additionally, I hypothesized that the residence times of aboveground biomass would be higher in swale locations compared to dune ridges. Furthermore, residence times of aboveground biomass would be shorter in the Pulse treatment as the plant community shifts to an annual and C\textsubscript{3} dominated community with higher litter quality (lower C:N).

**Methods**

**Study Site & Experimental Design**

This study was conducted within the Grassland Destabilization Experiment (GDEX) located at UNL’s Barta Brothers Ranch (BBR) in the eastern Nebraska Sandhills
Mean annual temperature at BBR is 8.1 °C and mean annual precipitation is 576 mm (Wang et al. 2009b), where 80% of precipitation falls during the growing season between April and September (Schacht et al. 2000). In general, around 90% of the landscape is comprised of upland dunes and dry interdunal areas (swales) dominated by warm-season, C₄ prairie grasses, while the remaining 10% is naturally subirrigated wet meadows and wetlands. The GDEX site itself is categorized as part of the 90% upland prairie, with no naturally subirrigated wet meadows. The dunes are composed of Holocene eolian sand deposits, which overlie Quaternary and/or Pliocene alluvial sand and silt (Wang et al. 2009a). The sand dune soils are mostly composed of Valentine fine sand soils with an average of 94% sand and 0.61% OM at 30 cm depth (Soil Survey Staff et al. 2013). Across the Nebraska Sandhills, the dunes are generally oriented from the northwest to the southeast, and dune heights can be as large as 40m high, 825m long, and 1220m wide (Bleed and Flowerday 1998). At BBR, dunes are generally smaller, ranging from 5-20m in height (Wang et al. 2009a) and slopes are 5-15%.

The dunes are covered by a mixed-grass prairie assemblage, where the soil properties and vegetation cover vary by topography. Interdunal swale locations have greater soil carbon content (0.51%) compared to dune slopes and ridges (0.33%) in the top 30cm, with slightly less sand in the top 10cm of swale locations (91.2% sand) compared to dune slopes and ridges (94.4% sand). The A horizons in dune swales extend to an average depth of 22.8cm (range 6-41cm), while A horizons on the dune ridges extend to an average depth of 11.8cm (range: 6-21cm; Wang et al. 2008). The interdunal
swales (15% of the landscape) contain a higher proportion of switchgrass (*Panicum virgatum*), bluegrass (*Poa pratensis*), blue grama (*Bouteloua gracilis*), and sedges (*Carex eleocharis*). Dune slopes and ridges (85% of the landscape) are composed of warm-season, C₄ grasses such as prairie sandreed (*Calamovilfa longifolia*) and sand bluestem (*Andropogon hallii*), which are generally located on south facing slopes, while the north slopes contain more cool-season, C₃ grasses such as needle and thread grass (*Hesperostipa comata*), prairie june grass (*Koeleria macrantha*), and the shrub leadplant (*Amorpha canescens*) (Schacht et al. 2000, Milby 2011).

The GDEX project was set up in 2004 with ten circular plots, 120m in diameter (1.1 hectares total area), which span the profile of one sand dune, from swale to dune top to swale. Within each plot, there were 22 subplots, with varying locations across elevation, aspect, and slope. Each of the ten plots was assigned to one of five management treatments, which range from continuous grass cover to bare sand. Treatments included two Control plots (with no grazing), two Grazed plots (deferred rotational grazing), two Press plots (no grazing, plant growth prevented with herbicide), two Pulse plots (no grazing), and two Aggressive plots (no grazing, vegetation killed with herbicide and disked; treatment not used in this analysis). Press and Pulse treatments were chosen to examine the response of the Sandhills grassland ecosystem to experimental vegetation disturbances and provide insights into the stability and resilience of the sand dune grasslands to permanent and periodic vegetation suppression (Bengtsson et al. 2003, Collins et al. 2011). Plots in the Pulse treatment are on a 3-year schedule, with vegetation killed with herbicide in the first year (reapplications to prevent regrowth),
followed by two years of recovery. Vegetation in the Pulse plots was killed (without physical disturbance) in 2005, 2008, 2011, and 2014, but allowed to recover during intervening years (see Chapter 1 for detailed methods). The Press treatment is similar to the Pulse treatment, but does not allow for vegetation recovery. Vegetation in the Press treatment plots was killed with herbicide in May 2005, with further reapplications as necessary to prevent vegetation regrowth (see Chapter 1 for detailed methods). Vegetation was kept dead from 2005-2010, at which point the Press treatment was discontinued because the plots were fully mobilized. The Press plots were subsequently planted with native shrubs and grasses beginning in 2011 to facilitate dune stabilization. Plots in the Grazed treatment were part of the deferred rotational grazing study at Barta Brother Ranch (Schacht et al. 2010, 2011). From 2004-2014, the plots in the DRG study pastures were grazed with 60-132 cow-calf pairs (1.5 AUE) over either three periods (2-14 days) or one period (30-42 days) during the grazing season (15 May – 15 October). Stocking rates were moderate, varying from 1.65-2.12 AUM ha⁻¹ (see Chapter 1 for more details).

**Sampling Procedure**

Aboveground biomass was sampled several times across the growing season (April-October) from 2005-2014. Although the sampling frequency changed from year to year, varying from every two weeks to once a month, aboveground biomass was sampled at least five times during the growing season within each year. In 2005, biomass was sampled 10 times, nine times in 2006, eight times in 2007, six times in 2008 and 2009, five times from 2010-2013, and six times in 2014. Control, Grazed, and Pulse
treatments were sampled continuously from 2005-2014. The Press treatments were sampled from 2005 through 2010, when the treatment was discontinued, as the plots had become blowing sand dunes.

Aboveground biomass samples were collected from one designated ridge (dune top) and one swale (interdunal) subplot in each plot. Exclusion cages were not used in the Grazed treatment plots, so the aboveground biomass responses are not estimates of ANPP like the Control treatment aboveground biomass. On each sampling date, aboveground biomass samples were hand clipped using two 0.25 m$^2$ sub-samples in each subplot location. On the next sampling date, biomass was collected from two more 0.25 m$^2$ sub-samples adjacent to the previous month’s sub-sample locations. Vegetation was sorted into six categories, roughly based on functional plant groups and photosynthetic components; 1) grass/sedge (monocots), 2) forbs, 3) shrub stems, 4) shrub leaves, 5) cacti, and 6) dead plant material. Biomass samples were then be oven-dried at 55 °C for a period of no less than seven days. All biomass samples were then weighed after drying to determine biomass weight.

Additionally, a small portion of aboveground grass biomass samples were selected to analyze differences in $^{13}$C between Control and Pulse treatments, which may reflect changes in plant community composition. Grass biomass leaf samples were selected from an early season (April-June) and late season (July-October) date within each year from 2006-2013 (2005 samples were not available and 2014 samples had not been collected at time of analysis). Early and late season dates were selected to coincide with peak biomass for C$_3$ (mid-June) and C$_4$ (late August) species in the Nebraska
Aboveground grass biomass sub-samples from each topographic location in each plot were dried, sub-samples were mixed together, and then ground using a Cyclone Sample Mill. Ground samples (n=119) were packed, weighed, and sent to the Stable Isotope Mass Spectrometry Lab at Kansas State University (Manhattan, KS). Ground grass biomass samples were analyzed for δ^{13}C with a CE 1110 elemental analyzer.

**Statistical Analysis**

Response variables include live forb, live grass, and total live (forb + grass), aboveground biomass to highlight the effects of climate variability (drought) and vegetation disturbances (Pulse and Grazed) on general plant community composition. I excluded shrubs and cacti biomass fractions from total live aboveground biomass. Occurrences of shrubs (rose, sand cherry, and leadplant) and cacti were sporadic. Shrub biomass skewed total live biomass distributions, and both shrub (0.00 – 7.58%) and cacti (0.00 – 7.91%) biomass fractions contributed little to total live biomass relative to grass and forb fractions. Dead biomass fractions were not partitioned into current year dead and previous year dead, so I was not able to directly estimate aboveground net primary production (Fahey and Knapp 2007) and instead used peak live biomass (forbs + grasses) as an estimate of ANPP. However, the dead biomass fractions were used to determine residence times of aboveground biomass.

I analyzed differences in peak aboveground live biomass and residence times of aboveground biomass ($RT = \frac{total\ live + dead}{total\ live}$), using a mixed effects model (JMP 11.0.0)
with treatment, topography, and year as fixed effects and the nesting of sub-samples, subplot, and plot as random effects. I selected peak biomass values by determining the date with the greatest aboveground live biomass for each specific subplot within plot, within each year. Although this method was biased towards higher values and blurs the timing of peak biomass, I was interested in producing an estimate of ANPP, which will vary inter annually, so I selected the specific dates with the highest total live aboveground biomass for each subplot-plot combination within each year. Although using aboveground live biomass underestimates ANPP because the recently senesced biomass fraction is left out (Fahey and Knapp 2007), in the Nebraska Sandhills, the current year senesced plant material accounts for 8.6 – 19.8% of ANPP (Mousel et al. 2011). Multiple comparisons between treatments, topographic positions, and years were done using Tukey’s HSD.

To run the mixed-model analysis on differences in peak live biomass, I estimated aboveground grass, forb, and dead biomass for swale locations in the Control for 2010, as all of the designated biomass sampling subplot locations were underwater. I used a simple linear regression model using the cumulative precipitation at peak production (Figure 2-1) to predict grass, forb, and dead biomass fractions at peak production for swale subplot locations in the Control in 2010. Linear regression models estimated the aboveground live grass \((y = 45.4333 + 0.2387\times X; R^2 = 0.23)\) better than the aboveground live forb \((y = 0.4992 + 0.0783\times X; R^2 = 0.18)\) or aboveground dead biomass \((y = 485.7244 – 0.3578\times X; R^2 = 0.14)\) at peak production in the swales of Control plots in 2010.
To determine shifts in the timing of peak biomass, and the relative contribution of grass and forb fractions to total live biomass, I used a mixed effects repeated measures model using treatments, topography, and month as fixed effects, and the subplot as the repeated subject. Multiple comparisons between treatments, topographic positions, and years were done using Tukey’s HSD. The Press treatment data was analyzed separately from the Control, Grazed, and Pulse treatment data because the Press treatment resulted in little to no live aboveground biomass. A similar mixed-model analysis (JMP 11.0.0) was conducted with just year and topography as fixed effects, to determine how quickly the aboveground dead biomass decomposed. Additionally, differences in the isotopic signature of $^{13}\text{C}$ in aboveground live grass biomass were analyzed using a mixed effects model with year, treatment, topography, and season as fixed effects. I used the analysis of the isotopic signature of $\delta^{13}\text{C}$ in aboveground live grass biomass to further determine plant community composition changes between the Control and Pulse treatment, and if the plant community changed over the course of the study.

**Results**

Aboveground live biomass (g m$^{-2}$) varied between treatments, topography, intra and inter annually from 2005-2014 (Figure 2-2). Across all treatments, total (forb + grass) live aboveground biomass was generally greater in swale locations than upland locations (Figure 2-2). In Control and Grazed treatments, the aboveground live grass biomass contributed more to the overall total live aboveground biomass; while in the Pulse treatment, the live grass biomass was reduced and the live forb biomass contributed more to overall total live aboveground biomass (Figure 2-2).
In the following sections, I detail the treatment and topographic differences in peak aboveground live forb, live grass, and total live biomass as well as the seasonal trajectories of the same components in undisturbed (Control) sand dunes and sand dunes with vegetation disturbance (Grazed and Pulse treatments). The peak biomass results presented in the following sections will be different from values presented earlier (Figure 2-2), where aboveground live biomass was averaged for each treatment and topographic position at each sampling date. The values presented in the following sections use the peak aboveground live biomass for each specific subplot within a plot as an estimate of ANPP, so I chose a variety of dates, instead of a single date within each year.

Additionally, I present isotopic evidence of plant community changes in the Control and Pulse treatments. Finally, to understand how aboveground biomass persists in the Sandhills grasslands, I describe the temporal decomposition of dead vegetation in the Press treatment as well as presenting residence times of aboveground biomass in the Control, Grazed, and Pulse treatments.

**Undisturbed Sand Dunes**

In this ten-year study, the aboveground total (forb + grass) live biomass, live grass biomass, and live forb biomass varied significantly between topographic positions (swales vs ridges) at peak production and within the growing season in the undisturbed Control plots (Table 2-1, Table 2-2). As expected, the mean peak total aboveground live biomass was strongly correlated with the mean cumulative precipitation at the time of peak biomass measurements (Adj. $R^2 = 0.697$), but swale locations were more responsive to increases in precipitation than ridge locations (Figure 2-3). Peak total live biomass
varied from 206.8 – 429.9 g m\(^{-2}\) in the swales and 62.1 – 192.1 g m\(^{-2}\) on the dune ridges. During the drought of 2012 (Figure 2-1), the lowest and second lowest peak total aboveground live biomass was recorded for the dune ridges and swales respectively, where the drought reduced aboveground biomass by 26% in swales and 54.5% on dune ridges (Figure 2-2, Figure 2-4). In general, the interdunal swale areas produced significantly greater peak total live biomass (294.7 ± 23.0 g m\(^{-2}\)) compared to the dune ridges (136.3 ± 23.0 g m\(^{-2}\)), ranging from 1.45 – 3.51 times higher than dune ridges (Table 2-1, Figure 2-4). This topographic difference in peak total live biomass was significant in eight out of the ten years of the study. Although not part of the peak biomass analysis, adding live shrub biomass resulted in similar topographic differences between swale (302.3 ± 18.5 g m\(^{-2}\)) and ridge (136.3 ± 8.0 g m\(^{-2}\)) locations. Across the growing season, aboveground total live biomass was significantly higher in swale locations in every month except April, and the total live biomass tended to peak in July and remain high through August in both ridge and swale locations (Table 2-2, Figure 2-5).

Similarly, the swales produced ~2.5 times more grass biomass (237.8 ± 21.8 g m\(^{-2}\)) during peak production compared to dune ridges (95.9 ± 21.8 g m\(^{-2}\)) (Table 2-1). Peak live grass biomass varied from 49.0 – 177.4 g m\(^{-2}\) on the dune ridges and 154.4 – 340.6 g m\(^{-2}\) in the swales (Figure 2-2). The peak live grass biomass component comprised an average of 70.4% of the peak total live biomass on dune ridges, and 80.7% in interdunal swales. Within the growing season, the relative contribution of the live grass fraction to total live biomass varies (swales: 78 – 95% and ridges: 61 – 83%), but because the live
grass biomass makes up a significant portion of the total live biomass, the differences between swales and ridges are similar to the total live biomass results presented earlier (Table 2-2). Aboveground live grass biomass in the swales was 6.2 and 3.7 times greater than ridge locations in April and October respectively, and 2.2 – 2.7 times higher from May through September (Figure 2-5). Peak live grass biomass occurred in July, and remained high in August, for both ridge and swale locations in the un-grazed Control plots (Figure 2-5).

There were no significant topographic or annual differences in peak live forb biomass (Table 2-1). The peak live forb biomass on the undisturbed dune ridges composed 29.6% (40.4 ± 6.8 g m\(^{-2}\)) of peak total live biomass and 19.3% (56.8 ± 6.8 g m\(^{-2}\)) in interdunal swales. From 2005-2014, peak live forb biomass ranged from 13.1 – 64.4 g m\(^{-2}\) on the dune ridges and 10.1 – 120.2 g m\(^{-2}\) in the interdunal swales. Seasonally, the live forb biomass peaked in August on both dune ridges and swales, with significantly higher forb biomass in swale locations compared to ridges during July, August, and September (Table 2-2, Figure 2-5).

**Grazing Effects**

The Grazed treatment had significant inter-annual effects on the peak aboveground total live, grass, forb biomass, as well as interacting with topographic position (Table 2-3). Additionally, grazing impacted aboveground total live, grass, and forb biomass seasonally (Table 2-4). Overall, from 2005-2014, the Grazed treatment (136 ± 16 g m\(^{-2}\)) had significantly lower total live biomass during peak production compared to the Control (215.46 ± 16.44 g m\(^{-2}\)), though these values are averaged over
topography. Although not significant (Table 2-3), the mean peak total live biomass in the Grazed treatment was higher in the swales (157.8 ± 23.1 g m\(^{-2}\); range: 111.5 – 207.4 g m\(^{-2}\)) compared to the dune ridges (113.5 ± 23.4 g m\(^{-2}\); range: 65.3 – 170.6 g m\(^{-2}\)). Similar to the Control, the lowest recorded peak production on dune ridges and second lowest in the swales was recorded during the drought of 2012 (Figure 2-1), where peak aboveground production was reduced by 24% in the swales and 42% on the dune ridges (Figure 2-2). The mean peak total live biomass in the Grazed treatment was only significantly lower than the Control in 2007, 2008, and 2011 (Figure 2-6). Similarly, aboveground live biomass across the growing season was significantly lower from May to October in the Grazed treatment compared to the Control (Figure 2-7). The total aboveground biomass in the Grazed treatment peaked in July, decreased by ~19% in August, and then increased by ~10% in September (Figure 2-7).

The mean peak live grass biomass in the Grazed treatment was only significantly lower than the Control in 2008, 2009, and 2011 (Figure 2-6). From 2005-2014, the peak live grass biomass made up 85.6% of the peak total live biomass in the swales and 82.0% of the peak total live biomass on the dune ridges. The peak live grass biomass in the Grazed treatment was higher in the swales (136.6 ± 19.7 g m\(^{-2}\); range: 88.7 – 196.1 g m\(^{-2}\)) compared to ridges (92.3 ± 19.8 g m\(^{-2}\); range: 50.0 – 160.7 g m\(^{-2}\)), but not significantly different (Figure 2-8). Grazed plots did have significantly lower grass biomass in the swales compared to the Control, but on dune ridges, there were not any differences between the Control and Grazed treatment (Figure 2-8). Across the growing season, the mean live grass biomass in the Grazed treatment peaked in July, and was significantly
lower than the Control from May to October (Figure 2-7). The seasonal trends of live
grass biomass in the Grazed treatment are similar to the total live biomass presented
earlier, because the grass fraction makes up 60 – 89% of mean monthly total live biomass
in the swales, and 34 – 90% on the dune ridges.

Mean peak live forb biomass did not differ significantly between the Control and
Grazed treatment within any year from 2005–2014. In the swales, the mean peak live
forb biomass varied from 2.7 – 47.6 g m⁻² and 0.5 – 53.4 g m⁻² on the dune ridges (Figure
2-6). Peak live forb biomass in the Grazed treatment comprised 1.5% – 31.9% of the
peak total live biomass. Topographically, the live forb biomass during peak production
was not significantly different between swale (21.4 ± 8.5 g m⁻²) and dune ridge locations
(21.5 ± 9.2 g m⁻²). Compared to the Control, mean live forb biomass in the Grazed
treatment was lower in both swale and ridges, although the only statistical difference was
in the swales (Figure 2-8). Seasonally, the monthly mean aboveground live forb biomass
in the Grazed treatment was only significantly lower than the Control during July and
August (Figure 2-7). Aboveground live forb biomass did tend to peak later in the
growing season during September, whereas the live forb biomass in the Control peaked a
month earlier in August (Figure 2-7).

Experimental Pulse Vegetation Disturbances

The experimental Pulse disturbance had significant effects on the peak
aboveground live biomass, with inter annual variation and interactions with topographic
position (Table 2-3). Averaged across the ten years, these treatment differences in
aboveground live forb, grass, and total biomass also varied by month within the growing
season (Table 2-4). Overall, from 2005-2014, the Control (215.5 ± 16.4 g m\(^{-2}\)) had significantly higher mean aboveground total live biomass during peak production (averaged over topography) compared to the Pulse (147.9 ± 16.4 g m\(^{-2}\)) treatment. However, the mean aboveground peak total live biomass in the Pulse was not significantly different from the Grazed treatment. Because of the experimental Pulse treatment, peak total live biomass was more variable, driven by the increases in the live forb biomass fraction in recovery years after the herbicide treatment (Figure 2-2, Figure 2-6). Herbicide treatments in 2005 and 2008 reduced the mean peak total live biomass to 24.3 and 8.3 g m\(^{-2}\), while the herbicide treatments in 2011 and 2014 were less effective, reducing the mean total live biomass to only 111.7 and 83.3 g m\(^{-2}\) respectively (Figure 2-6). If herbicide treatment years are excluded (2005, 2008, 2011, and 2014), peak total live biomass averaged 242.4 ± 43.0 g m\(^{-2}\) (range: 178.8 – 399.0 g m\(^{-2}\)) in the swales and 160.9 ± 27.6 g m\(^{-2}\) (range: 92.7 – 284.1 g m\(^{-2}\)) on the dune ridges. From 2005–2011, the mean peak total live biomass in the Pulse treatment was either significantly higher (2006) or significantly lower (2005, 2007–2008, 2010–2011, 2014) than the Control, but from 2012–2013, there were no significant differences between any of the treatments (Figure 2-6). Seasonally, the mean total aboveground live biomass in the Pulse was significantly lower than the Control from May to October (Figure 2-7). Interestingly, the mean total live biomass in the Grazed treatment was only significantly greater than the Pulse treatment during May, June, and October, when the live forb biomass in the Pulse treatment was low (Figure 2-7).
One of the largest effects of the Pulse treatment was reducing the live grass biomass fraction. The mean peak live grass biomass fraction was consistently low in the Pulse treatment from 2005–2014, varying from 7.3 – 104.5 g m$^{-2}$, and was significantly lower than the Control and Grazed treatment in every year (Figure 2-6). Averaged over the ten years, the peak live grass biomass fraction comprised 23.2% of the total live biomass in the swales and 30.6% on the dune ridges. Unlike the Control and Grazed treatment, peak live grass biomass did not significantly differ between swale (42.2 ± 19.7 g m$^{-2}$) and dune ridge (34.8 ± 19.7 g m$^{-2}$) locations in the Pulse treatment (Figure 2-8). Peak live grass biomass was significantly lower in the Pulse swales compared to the Control and Grazed treatment (Figure 2-8). The seasonal trend of the aboveground live grass biomass differed in the Pulse treatment compared to the Control and Grazed (Table 2-4). The aboveground live grass biomass was significantly lower than the Control and Grazed treatment over most of the growing season, from May to October. Additionally, live grass biomass peaked earlier in the season in June, and contributed 15 – 88% of the total live biomass in the swales and 26 – 85% on the dune ridges, with the greatest contributions occurring early in the growing season in April and May (Figure 2-7).

With the reduction in the live grass biomass fraction in the Pulse treatment, the contribution of the live forb biomass fraction to the total live biomass increased at both peak production and across the growing season (Table 2-3, Table 2-4). In the first recovery year after herbicide treatments (2006, 2009, and 2012), peak live forb biomass in the Pulse treatment ranged from 3.8 – 17.0 times higher than the Control (Figure 2-6). Additionally, the drought in 2012 resulted in a similar response, with peak live forb
biomass in the next year (2013) remaining high (Figure 2-6). The peak live forb biomass in the Pulse treatment was significantly greater in the swales compared to the ridges, and was also significantly higher than Control and Grazed treatments at both ridge and swale locations (Figure 2-8). The peak live forb biomass in the Pulse swales composed, on average, 76.8% (139.9 ± 8.8 g m⁻²) of the peak total live biomass and 69.4% (78.8 ± 8.8 g m⁻²) on the dune ridges. The aboveground live forb biomass fraction did not differ significantly between the Pulse, Control, and Grazed treatments in April, May, June, or October (Table 2-4, Figure 2-7). However, from July to September, the live forb biomass in the Pulse treatment increased to 66 – 77 g m⁻² and made up 73 – 80% of the total biomass, enough to offset the reduced live grass biomass fraction during those months and produce total aboveground live biomass that was not significantly different from the Grazed treatment (Figure 2-7).

**¹³C Isotopic Analysis**

There were no topographic differences in the mean ¹³C signature between Control and Pulse treatments in swale locations, so only the ridge locations, where greater differences occurred, are presented here. Isotopic analysis of grass leaf tissue (δ¹³C ‰) from ridge locations in the Control and Pulse treatments showed significant main effects of year, season, treatment, and interactions of treatment × year, and treatment × year × season (Table 2-5). Overall, the Pulse treatment had a significant effect, where the mean isotopic signature of grass biomass from dune ridges (averaged between early and late season) in the Pulse treatment (-23.00 ± 0.81 ‰) was lighter over the nine years compared to the Control (-18.11 ± 0.79 ‰). From 2005-2013, the δ¹³C isotopic
signature in grass leaf tissue was significantly heavier in the Control compared to the Pulse treatment in all years except for 2007 and 2010. There was a three-way interaction between treatment, year, and season (Table 2-5). In general, the isotopic signature of $^{13}\text{C}$ in grass biomass in the Control was heavier than the Pulse treatment, and the signature generally was lighter in the early season and heavier in the late season (Figure 2-9). The Pulse treatment did not generally follow the seasonally pattern as in the Control (except in 2009, 2010, and 2013), and in most years the signature was not significantly different between seasons (Figure 2-9).

I used the isotopic signatures of grass leaf tissue in both swale and ridge locations in the Control and Pulse treatments to estimate the proportion of C$_4$ species in the grass community (Tieszen and Boutton 1989, Boutton 1996), assuming isotopic signatures of -$28\%$ for C$_3$ and -$12\%$ for C$_4$ grass species (Dawson et al. 2002). Additionally, I estimated the total C$_4$ community composition at peak production by including the contribution of forbs (assuming 100% C$_3$) and weighting the values based on the relative occurrence of swales (15%) and dune ridges (85%) across the landscape (Stephenson 2010). On the un-grazed Control sand dunes, grass leaf tissue $^{13}\text{C}$ signatures averaged -$18.1 \pm 0.8\%$ on dune ridges and -$25.1 \pm 0.7\%$ in the swales during the late season, when peak production occurred (Figure 2-5, Figure 2-7). Using these values, I estimate the grass community to be 69.2% C$_4$ on the un-grazed dune ridges, 21.5% in the interdunal swales, and 62.1% C$_4$ grass across the landscape (Table 2-6). Assuming the peak live forb biomass fraction in swales (19.3%) and ridges (29.6%) is 100% C$_3$, the plant community is 48.8% C$_4$ on un-grazed dune ridges, 14.9% in the interdunal swales
and 44.6% C₄ across the landscape (Table 2-6). In the Pulse treatment, grass leaf tissue

\(^{13}\text{C}\) signatures averaged -26.2 ± 0.5 \(^{0}/\text{oo}\) on the ridges and -24.0 ± 0.8 \(^{0}/\text{oo}\) in the swales during the early season, when peak production of the grass fraction occurred (Figure 2-7). Using these values, I estimated the grass community to be 11.4% C₄ on the Pulse treatment dune ridges, 24.9% in the interdunal swales, and 13.4% C₄ grass across the landscape. Assuming the peak live forb biomass fraction in swales (76.8%) and ridges (69.4%) is 100% C₃, the plant community is 3.8% C₄ on Pulse treatment dune ridges, 5.8% in the interdunal swales and 3.9% C₄ across the Pulse treatment landscape.

**Persistence of Aboveground Biomass**

The Press treatment shifted almost all of the aboveground live vegetation into the dead biomass fraction, and vegetation regrowth was prevented from 2005–2010 (dunes were mobilized by 2010). A mixed-model analysis (Adj. R\(^2\) = 0.76; Obs = 290) incorporating year, topography, and their interaction, on the remaining aboveground dead biomass indicated significant effects of year (p<0.0001), but no significant effects of topography (p=0.1054) or topography × year interactions (p=0.5767). When the Press treatment was imposed in April 2005, the mean (±1 SE) aboveground dead biomass was 189.5 ± 32.2 g m\(^{-2}\) (Figure 2-10 inset). By April 2006, a full year after the initial treatment, the dead biomass declined by 9.3% (171.9 ± 44.2 g m\(^{-2}\)). After a second full year of preventing vegetation growth (April 2007), the aboveground dead biomass declined sharply to an average of 32.8 ± 6.4 g m\(^{-2}\), a 80.9% decrease from the previous year, and 17.3% of the original aboveground dead biomass remained after two years (Figure 2-10 inset). The dead biomass after a third year (April 2008; 15.9 ± 4.7 g m\(^{-2}\))
was reduced by 51.5% from the previous year, with 8.4% of the original aboveground dead biomass remaining after three full years. By the end of the biomass sampling in 2009 (five growing seasons), the aboveground dead biomass was completely gone, and erosion rates began to increase sharply (Figure 2-10). Additionally, the remaining aboveground dead biomass in the Press treatment was correlated with the amount of soil erosion (Figure 2-10 inset). As decomposition occurred and the remaining aboveground dead biomass was reduced below 50 g m$^{-2}$, soil erosion rates increased. After the A horizon was lost (mean depth = 11.8 cm; Wang et al. 2008), erosion rates rapidly increased until an average of ~50cm (range = +63.6 to -215.7 cm) of soil was lost across all topographic locations in the Press treatment plots by the end of 2014 (Figure 2-10).

The residence time of aboveground biomass ($RT = \frac{\text{total live} + \text{dead}}{\text{total live}}$) was analyzed using a mixed effects model with treatment, topography, and the interaction as fixed effects and year as a random effect. The Pulse treatment years (2005, 2008, 2011, and 2014) were omitted as most, if not all, of the biomass was dead. Additionally, an unplanned fire occurred in a single Control (plot 4) and Grazed plot (plot 10) in the spring of 2013, so I removed the 2013 and 2014 values of those plots. The mixed model analysis (Adj. $R^2 = 0.31$; Obs = 200) indicated a significant effect of treatment ($p<0.0001$) and a significant treatment $\times$ topography interaction ($p = 0.0115$).

Aboveground biomass in the Control had a mean residence time of $2.4 \pm 0.2$ years, $2.1 \pm 0.2$ years in the Grazed treatment, and $1.6 \pm 0.2$ years in the Pulse treatment. The residence times were significantly longer in the Control compared to the Grazed ($p = 0.0347$) and Pulse ($p<0.0001$), and significantly longer in the Grazed compared to the
Pulse treatment ($p = 0.0036$). The mean residence time in the swale locations for the Control ($2.5 \pm 0.2$ years) was significantly longer than both Grazed ($2.0 \pm 0.2$ years) and Pulse ($1.9 \pm 0.2$ years) treatments (Figure 2-11). In ridge locations, the residence time of aboveground biomass in the Pulse treatment ($1.4 \pm 0.2$ years) was significantly shorter compared to the Control ($2.3 \pm 0.2$ years) and Grazed treatment ($2.3 \pm 0.2$ years; Figure 2-11). Residence times of aboveground biomass were not significantly different between swales and ridges in the Control and Grazed treatment, but in the Pulse treatment the residence times were significantly shorter ($p = 0.0270$) on ridges compared to the swales (Figure 2-11).

**Discussion**

Other studies conducted at the Barta Brothers Ranch validate my approach in estimating the primary production of the Sandhills (Table 2-6). In deferred rotational grazing studies with altered time of grazing, the mean aboveground primary production (measured in exclosures in the year after grazing) ranged from $171.0 – 175.9$ g m$^{-2}$ across the pasture, with dune ridges varying from $152.6 – 176.0$ g m$^{-2}$, and $234.1 – 282.3$ g m$^{-2}$ in interdunal swales during a similar timeframe from 2001-2008 (Stephenson et al. 2015). These results are similar to the un-grazed Control sand dunes, where mean aboveground live biomass was $136.3$ g m$^{-2}$ (range: $62.1 – 192.1$ g m$^{-2}$) on the dune ridges (Table 2-6). However, biomass of the interdunal swales was greater (mean: $294.7$ g m$^{-2}$; range: $206.8 – 429.9$ g m$^{-2}$), likely due to grazing impacting swale locations disproportionately. Although the values for aboveground live biomass were smaller on dune ridges, their estimates included shrub components, which more accurately reflect aboveground
biomass production (Stephenson et al. 2013). In spite of these differences, I recorded similar trends in total aboveground live biomass in the overlapping study years from 2005 – 2008. Peak aboveground live biomass in swale locations in this study followed the same pattern of a decline in 2006, and subsequent increases in 2007 and 2008 (Figure 2-2, Figure 2-4). The pattern from the dune ridge locations was slightly different though, where peak aboveground live biomass was lower in 2005 in this study (114 g m\(^{-2}\)) compared to theirs (~200 g m\(^{-2}\); Stephenson et al. 2013), but from 2006 – 2008, I recorded the same year on year increase (Figure 2-2, Figure 2-4). I also recorded similar aboveground forb biomass in 2005, 2006, and 2008 (range 30 – 45 g m\(^{-2}\)), and the increase in forb biomass in 2007 (79.9 g m\(^{-2}\); Figure 2-6) was close to Stephenson et al. (2013) recorded forb biomass values of ~85 g m\(^{-2}\).

Although the aboveground live biomass (ANPP estimate) was measured at point locations on the ridges of sand dunes and in the interdunal swales, I weighted these values by the relative proportion of the topographic locations across the Sandhills landscape to estimate the productivity across the entire dune landscape. In the upland prairie areas of the Nebraska Sandhills, the interdunal swales cover an average of 15% of the landscape, while the slopes and ridges of the sand dunes cover an average of 85% of the landscape (Schacht et al. 2000, Stephenson et al. 2015). Using the mean swale and ridge values for Control and Grazed treatments presented earlier, I estimated that the landscape weighted peak production on Grazed sand dunes was 120.5 g m\(^{-2}\), while ungrazed Control sand dunes produced 160.1 g m\(^{-2}\) (Table 2-6). These estimates of productivity across the landscape are similar to ranges (119 – 150 g m\(^{-2}\)) from other
grazing studies (Mousel et al. 2011, Redden 2014) but lower than modeled estimates (Sala et al. 1988, Burke et al. 1997, Mangan et al. 2004, Bradford et al. 2005). However, my estimates are weighted towards ridge locations, without including north and south slope values, which produce more aboveground biomass than the ridges but less than the swales (Schacht et al. 2000, Stephenson et al. 2015). I also left out the shrub fraction and the current year’s dead biomass, both of which contribute to aboveground net primary productivity.

**Grazing Impacts in Interdunal Swales**

In the Sandhills, cattle tend to favor swale, or interdunal positions between sand dunes, over the dune ridges (Stephenson et al. 2013). This is mainly due to the greater biomass and higher proportion of C\textsubscript{3} species (85% in the Control swales; Schacht et al. 2000), which have higher leaf tissue N concentrations (Gibson 2009). My data supports this conclusion, with significantly lower live grass biomass in the swales of the Grazed treatment compared to the Control, and more importantly, with no differences in the aboveground live grass biomass on ridge locations between the Grazed and Control (Figure 2-8). Additionally, although the differences were not statistically significant, the residence time of aboveground biomass was longer at ridge locations compared to swale locations in our Grazed treatment (Figure 2-11 inset). This trend was opposite of the Control, where residence times of aboveground biomass were longer at swale locations compared to the dune ridges. Together, this evidence supports the notion that cattle tend to favor grazing the interdunal swale locations over sand dune ridges. Although grazing alters ecosystems processes (Li and Guo 2014), the impacts are greater in interdunal
swale areas. Because there were no differences in aboveground live grass biomass and
residence time of biomass between Control and Grazed ridges, concerns about exposing
the sandy soils to erosion on sand dune ridges may be overstated. However, one caveat is
that in the Sandhills, grazing is relatively moderate and well-managed (Volesky et al.
2004), and heavier, poorly managed grazing could still facilitate soil erosion (Vermeire et

**Aboveground Vegetation and Sand Dune Stability**

The purpose of the experimental Pulse treatment was to provide insight into the
ability of the sand dunes to recover under strong, periodic disturbances (Bengtsson et al.
2003, Collins et al. 2011). Application of the herbicide Pulse treatment was more
effective in some years than others (Figure 2-2, Figure 2-6), but overall, the treatments
successfully disturbed the aboveground vegetation and altered the plant community.
Although the Pulse treatment did significantly reduce the total aboveground live biomass
compared to the Control, it was not significantly different from the Grazed treatment
(Table 2-3, Table 2-4). This treatment caused a shift in community composition, with the
forb biomass fraction accounting for an average of 74% of the peak live biomass, and 70 –
80% of the live biomass across the growing season (Figure 2-6, Figure 2-7), and
reduced the live grass biomass so it only accounted for 26% of the peak total live
biomass. Peak production in the Pulse treatment occurred on average during September,
where the Control and Grazed treatments occurred in July (Figure 2-7), supporting a shift
in community composition.
I also recorded a shift in the leaf tissue $^{13}$C signature of the grass biomass fraction. The isotopic signature of grass leaf tissue ($\delta^{13}$C $^0/_{_{00}}$) from ridge locations in the Pulse (-23.0 ± 0.8 $^0/_{_{00}}$) were significantly lighter than the Control (-18.1 ± 0.8 $^0/_{_{00}}$; which are averaged over early and late season), indicating a shift in the grass community from 61% C$_4$ to 31% C$_4$ species (Figure 2-9). Furthermore, if I use swale and ridge isotopic signatures from peak production in the Control (late season) and Pulse (early season), add in the contribution of forbs (assuming 100% C$_3$), and weight topography by the relative occurrence of swale and ridge locations, the plant community in the Pulse treatment has shifted from 44.6% C$_4$ (Table 2-6) to 3.9% C$_4$. Additionally, the residence time of dead biomass in the Pulse treatment (1.6 ± 0.2 years) was shorter than the Control (2.4 ± 0.2 years), supporting the shift to an annual, weedy plant community which generally has higher litter quality (lower C:N; Wedin 2004). Differences in the tissue N concentrations of the grass biomass were marginally significantly greater (p=0.0896) in the Pulse treatment (1.61 ± 0.06 %N) than the Control (1.34 ± 0.07 %N). Despite these large shifts in community composition, the increase in live forb biomass offset a large portion of the lost live grass biomass.

The increase in the live forb fraction and the shift to a C$_3$ dominated grass community alters both the aboveground plant cover and the belowground productivity (Eisenhauer 2011, Avolio et al. 2014). Although I did not measure plant cover, I observed an increase in open spaces across the sand dunes. The sparsely distributed forb vegetation, along with a probable decrease in the fibrous root system due to the difference in root structure and function of annual forb species (Nippert and Knapp 2007,
Angelo and Pau 2015), increases the potential for wind erosion (Li et al. 2007).

Additionally, although annual plant communities have lower N use efficiency (Wedin 1995), there are abundant N sources within the Pulse treatment (see Chapter 3). Annual and C₃ dominated plant communities allocate a greater proportion of their biomass aboveground than belowground, decreasing the ability of the root-soil complex to buffer the sandy soils against erosion (Bardgett et al. 2014). Although it seems that the sand dunes are resilient to the Pulse treatment, there have only been three Pulse cycles, and a longer time-frame may be needed to observe a regime shift (Mangan et al. 2004). In fact, in the spring of 2015, small blowouts on the ridges of the Pulse plots occurred (Wedin, unpublished data). The question remains though, will these blowouts erode away the rest of the sand dune (Stubbendieck et al. 1989), or are the swale locations sufficiently buffered against this disturbance?

Aboveground vegetation in semi-arid and arid grasslands is hypothesized to provide protection against wind erosion (Stubbendieck et al. 1989), with decreases in vegetation cover leading to increased erosion (Vermeire et al. 2005, Li et al. 2007, 2009). I used the decomposition of Press treatment dead biomass, and the residence times of aboveground dead biomass in the other treatments, to determine how long aboveground biomass remains in this grassland ecosystem, potentially buffering against wind erosion. I found that only about 10% of the aboveground dead biomass was lost after one year in the Press treatment, but in the second year the litter was rapidly lost and was reduced to just 17% of the initial standing dead (Figure 2-10). Similarly, I calculated residence times of aboveground biomass in the other treatments (Figure 2-11) and found residence
times that varied from 1.35 years (ridge locations in Pulse) to 2.52 years (swale locations in Control).

Together, these two lines of evidence suggest that the aboveground litter in the Nebraska Sandhills persists for about 2-3 years (Potvin and Harrison 1984), with the second year of decomposition being critically important for litter loss. These values fit in the spectrum of residence times across other temperate grasslands in the Great Plains. In the shortgrass steppe, where ANPP and aboveground dead biomass equilibrate at 40 – 75 g m\(^{-2}\) yr\(^{-1}\) and 90 g m\(^{-2}\) yr\(^{-1}\) respectively, indicating a residence times are estimated to be 2.5, and in the tallgrass prairie ANPP (410 g m\(^{-2}\) yr\(^{-1}\)) and aboveground dead biomass (>1000 g m\(^{-2}\) yr\(^{-1}\)) equilibrate after 3 years (Knapp and Seastedt 1986, Tilman 1987, Köchy and Wilson 1997, Knapp et al. 1998, Lauenroth and Burke 2008). In the Press treatment, over 90% of the aboveground dead biomass decomposed after three years, yet soil erosion rates did not increase until the fifth dead year (Figure 2-10). Erosion of the top 10 cm of soil (A horizon; (Wang et al. 2008) occurred slowly, and then rapidly increased after losing the dense root-soil complexes in the A horizon. Although aboveground vegetation does reduce wind erosion rates (Li et al. 2005), it is likely that the legacy of the C\(_4\) grasslands and the continued functioning of the belowground processes is just as important in buffering wind erosion rates (see Chapter 4).

**Resilience to Drought**

Climate variability is a key driver of ecosystem processes in grasslands (Fay et al. 2003), with droughts in the Nebraska Sandhills being a relatively common disturbance. The worst drought in 117 years occurred in 2012 (Figure 2-1), increasing daily mean
temperatures by 1.9 – 6.1 °C during the summer months, and reducing precipitation to 257mm, which is 45% of the long-term average (Mallya et al. 2013, Hoerling et al. 2014). At peak production in 2012, the mean total live aboveground biomass was ~35% lower (averaged across topography) in the un-grazed Control sand dunes. I also recorded increased residence times of aboveground biomass (Figure 2-11) and isotopic signatures that shifted towards a C₄ dominated grass community (Figure 2-9). An interesting effect is how topographic positions on the sand dunes respond differently to drought and water availability (Pennington and Collins 2007, Istanbulluoglu et al. 2012, Hamilton 2013). Aboveground biomass during peak production is more highly correlated to cumulative precipitation in the interdunal swales than the dune ridges (Figure 2-3). However, I recorded a greater relative reduction in the peak total live biomass on the dune ridges (-54.5%) compared to the interdunal swales (-26.0%; Figure 2-4). Despite the severe drought in 2012, the sand dune grassland recovered in 2013 and 2014 (Figure 2-6), with peak total live biomass reaching average levels on dune ridges, but interdunal swales remained below average (Figure 2-4). Similarly, the residence times of aboveground dead biomass in 2013 and 2014 returned to the ten-year average on the sand dune ridges, but in the interdunal swales, the residence times remained significantly higher (Figure 2-11).

The differential responses of topographic positions provides a unique opportunity to determine how these sand dune grasslands function. Dune ridges serve as recharge zones for groundwater. While not sub-irrigated, in some years the interdunal swales can act as discharge zones producing wetlands or shallow lakes (Wang et al. 2008, 2009b).
The dynamic nature of the groundwater affects soil water availability, and can affect grassland responses to disturbances on a variety of timescales (Gosselin et al. 2006, Sridhar et al. 2006a). Interdunal swales in the un-grazed Control plots produced 2.2 times more peak total live biomass (+158.4 g m$^{-2}$), displayed greater proportions of C$_3$ species (85% vs. 51%), and had longer aboveground biomass residence times (Table 2-6). Aboveground biomass equilibrated after two to three years, and fire and drought seem to be short-term perturbations. With short residence times, fire may not be a critical component in this ecosystem to regulate litter accumulation. The absence of fire in this region will most likely impact the ecosystem in other ways, such as facilitating woody encroachment of eastern red cedar (Eggemeyer et al. 2006, 2009). Additionally, because swales (14.9% C$_4$) and dune ridges (48.8% C$_4$) have different C$_3$/C$_4$ community compositions (Table 2-6), they will physiologically respond differently to drivers of global change (Knapp et al. 2015). The different traits associated with grassland C$_3$ and C$_4$ species (Craine et al. 2002, Tjoelker et al. 2005) indicates that they will respond differently to drought and precipitation (Craine et al. 2011), N availability (Wedin and Tilman 1993), and increased atmospheric CO$_2$ concentrations (Curtis et al. 1999). Understanding the differences between swale and ridge communities, along with dune slopes, is critical to predict grassland productivity, carbon balance, evapotranspiration, and responses to climate change.

In general, the un-grazed sand dune grasslands were resilient to severe drought, recovering to the ten-year averages in a year or two. This is not surprising as the Sandhills and other temperate grasslands recovered from the chronic Dust Bowl droughts
of the 1930’s. The Dust Bowl droughts reduced plant biomass by 20 – 95% (Weaver and Albertson 1936), yet by 1945, much of the grasslands had recovered, albeit with large shifts in plant community composition (Albertson and Weaver 1944, 1946). Similar to other temperate grasslands, the Nebraska Sandhills are resilient to drought, as they have co-evolved with climate variability (Stubbendieck and Tunnell 2008). It is important to understand not just differing topographic responses to disturbance, but interactions of grazing, management, and climate change impacts predicted for the region (Sala et al. 2012, Polley et al. 2013, Rossman et al. 2014).

**Conclusion**

The production of aboveground biomass in the Nebraska Sandhills is resilient to both short-term, severe drought, moderate grazing, and experimental vegetation disturbances. Aboveground biomass in the Sandhills produced an average of 160.1 g m\(^{-2}\) on un-grazed dunes (ANPP estimate). In general, the grass component (71.9%) dominated total aboveground live biomass on un-grazed sand dunes, with interdunal swales producing more than two times the biomass as dune ridges. I estimated the residence time of dead biomass to be 1.9 – 2.5 years, depending on topographic position and grazing. A severe drought in 2012 reduced aboveground live biomass by an average of 35%, but biomass recovered to average levels in one to two years. Experimental Pulse disturbances reduced aboveground biomass and shifted community composition to an annual and C\(_3\) grass dominated plant community, with shorter residence times of aboveground biomass. After three cycles of Pulse disturbances, the sand dunes are still intact, although observational evidence suggest a threshold is approaching. My results
demonstrate the resilience of the Nebraska Sandhills to short-term, severe drought and experimental Pulse vegetation disturbances. However, the sand dunes were successfully mobilized in 4-5 years with no vegetation growth (Press), demonstrating the time lag associated when the grassland ecosystem shifts from a stabilized to a mobilized state. My results also show chronic pulse disturbances have the ability to alter the plant community structure, function, and on a longer times scale (>10 years), may ultimately shift the sand dune grassland into a mobilized state.
Figures & Tables

Table 2-1. Mixed-model ANOVA summaries for differences in annual peak aboveground live forb, grass, and total (forb + grass) biomass in Control plots from 2005-2014. Bolded numbers indicate statistical significance (p<0.05), and italicized numbers indicate marginal significance (p<0.10).

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<th>Adj. R²</th>
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Table 2-2. Mixed-model ANOVA summaries for differences in aboveground live forb, grass, and total (forb + grass) biomass by month and topography. Data are from Control plots from 2005-2014.

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<th>Adj. R²</th>
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<td>&lt;0.0001</td>
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</tbody>
</table>
Table 2-3. ANOVA summaries for the differences in live aboveground forb, grass, and total live (forb + grass) biomass at peak biomass within each year. Comparisons are between treatments (Control, Grazed, Pulse), topography (Swale, Ridge), and years (2005-2014). Bolded numbers indicate statistical significance (p<0.05).

<table>
<thead>
<tr>
<th>Adj. R²</th>
<th>Forb</th>
<th>Grass</th>
<th>Total Live</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Year</td>
<td>3.52</td>
<td>0.0005</td>
<td>6.73</td>
</tr>
<tr>
<td>Trt</td>
<td>52.25</td>
<td>0.0002</td>
<td>21.45</td>
</tr>
<tr>
<td>Topo</td>
<td>12.98</td>
<td>0.0112</td>
<td>16.09</td>
</tr>
<tr>
<td>Year × Trt</td>
<td>4.04</td>
<td>&lt;0.0001</td>
<td>2.97</td>
</tr>
<tr>
<td>Year × Topo</td>
<td>0.79</td>
<td>0.6237</td>
<td>2.38</td>
</tr>
<tr>
<td>Trt × Topo</td>
<td>6.42</td>
<td><strong>0.0320</strong></td>
<td>6.23</td>
</tr>
<tr>
<td>Year × Trt × Topo</td>
<td>0.52</td>
<td>0.9478</td>
<td>1.0275</td>
</tr>
</tbody>
</table>
Table 2-4. ANOVA summaries for the differences in live aboveground forb, grass, and total live (forb + grass) biomass within a growing season. Comparisons are between treatments (Control, Grazed, Pulse), topography (Swale, Ridge), and months (April–October). Bolded numbers indicate statistical significance (p<0.05).

<table>
<thead>
<tr>
<th>Source</th>
<th>Forb</th>
<th>Grass</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adj. R²</td>
<td>0.29</td>
<td>0.68</td>
<td>0.51</td>
</tr>
<tr>
<td>Month</td>
<td>27.57</td>
<td>&lt;0.0001</td>
<td>79.80</td>
</tr>
<tr>
<td>Trt</td>
<td>13.77</td>
<td>&lt;0.0001</td>
<td>64.06</td>
</tr>
<tr>
<td>Topo</td>
<td>6.79</td>
<td>0.0103</td>
<td>56.48</td>
</tr>
<tr>
<td>Month × Trt</td>
<td>5.87</td>
<td>&lt;0.0001</td>
<td>15.52</td>
</tr>
<tr>
<td>Month × Topo</td>
<td>3.02</td>
<td>0.0061</td>
<td>6.18</td>
</tr>
<tr>
<td>Trt × Topo</td>
<td>3.23</td>
<td>0.0431</td>
<td>16.53</td>
</tr>
<tr>
<td>Month × Trt × Topo</td>
<td>0.98</td>
<td>0.4750</td>
<td>2.80</td>
</tr>
</tbody>
</table>
Table 2-5. ANOVA summary of δ¹³C values from grass leaf biomass samples at dune ridge locations. Data were from Control and Pulse treatments from 2005-2013. Bolded numbers indicate statistical significance (p<0.05).

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2.79</td>
<td>0.0256</td>
</tr>
<tr>
<td>Season</td>
<td>18.58</td>
<td>0.0002</td>
</tr>
<tr>
<td>Treatment</td>
<td>18.57</td>
<td>0.0344</td>
</tr>
<tr>
<td>Year × Season</td>
<td>1.40</td>
<td>0.2463</td>
</tr>
<tr>
<td>Year × Treatment</td>
<td>4.68</td>
<td>0.0015</td>
</tr>
<tr>
<td>Season × Treatment</td>
<td>0.09</td>
<td>0.7697</td>
</tr>
<tr>
<td>Year × Treatment × Season</td>
<td>4.92</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

Adj. R² = 0.87  Obs = 61
Table 2-6. Summary of peak aboveground biomass components for the un-grazed Control ridge and swale locations, and landscape weighted totals. I am presenting the means (±1 SE) and ranges for aboveground live grass, forb, shrub, aboveground dead and total live fractions, as well as the residence time of aboveground biomass (2005-2014). For the live grass and forb fractions, I also present the percentage of the total peak live aboveground biomass. Additionally, I present the estimated percent C₃ and C₄ grass fractions (using late season ¹³C isotopic signatures) and the total estimated percent C₃ and C₄ of the grassland community (assuming all forbs are C₃). Finally, I present the total landscape weighted values, where I assumed ridge locations comprised 85% of the landscape and swale locations 15% (Stephenson 2010).

<table>
<thead>
<tr>
<th></th>
<th>Grass (g m⁻²)</th>
<th>Forb (g m⁻²)</th>
<th>Shrub* (g m⁻²)</th>
<th>Total Live (g m⁻²)</th>
<th>Dead (g m⁻²)</th>
<th>Residence Time</th>
<th>C₃ (%)</th>
<th>C₄ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridge</td>
<td>95.9 ± 21.8</td>
<td>40.4 ± 6.8</td>
<td>0</td>
<td>136.3 ± 23.0</td>
<td>169.9 ± 15.9</td>
<td>2.3 ± 0.2</td>
<td>Grass: 30.8</td>
<td>69.2</td>
</tr>
<tr>
<td></td>
<td>48.9 – 177.4</td>
<td>13.1 – 64.4</td>
<td>0 – 0</td>
<td>62.1 – 192.1</td>
<td>90.9 – 303.8</td>
<td>1.7 – 3.9</td>
<td>Total: 51.2</td>
<td>48.8</td>
</tr>
<tr>
<td></td>
<td>70.4%</td>
<td>29.6%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Swale</td>
<td>237.8 ± 21.8</td>
<td>56.8 ± 6.8</td>
<td>4.9 ± 2.0</td>
<td>294.6 ± 23.0</td>
<td>360.6 ± 37.8</td>
<td>2.5 ± 0.2</td>
<td>Grass: 78.5</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>154.4 – 340.6</td>
<td>10.1 – 120.2</td>
<td>0.0 – 19.8</td>
<td>206.8 – 429.9</td>
<td>191.8 – 600.3</td>
<td>1.5 – 5.7</td>
<td>Total: 85.1</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>80.7%</td>
<td>19.3%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Total**</td>
<td>117.2</td>
<td>42.9</td>
<td>0.7</td>
<td>160.1</td>
<td>198.5</td>
<td>2.4</td>
<td>Grass: 37.9</td>
<td>62.1</td>
</tr>
<tr>
<td>(landscape weighted)</td>
<td>71.9%</td>
<td>28.1%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Total: 55.4</td>
<td>44.6</td>
</tr>
</tbody>
</table>

*Shrub component not used in analysis to estimate total aboveground live biomass. See Stephenson et al. (2013, 2015) for complete shrub information.

**Does not include naturally sub-irrigated meadows
Figure 2-1. Cumulative precipitation (mm) from 2004–2014, recorded near the GDEX site at Barta Brother’s Ranch in the Central Nebraska Sandhills. Data obtained from the High Plains Regional Climate Center (High Plains Regional Climate Center 2015).
Figure 2-2. Mean aboveground live forb, grass, and total live (forb + grass) biomass (g m\(^{-2}\)) by topographic location (Swale and Ridge) in Control (A & B), Grazed (C & D), and Pulse (E & F) treatments for each sampling date from 2005-2014. Arrows denote the application of the Pulse herbicide treatment. Samples in swale locations in Control plots in 2010 are missing because subplots were inundated with water, while samples in April of 2012 in the Grazed treatment were not clipped.
Figure 2-3. Linear regression (± 95% CI) of the mean (±1 SE) peak total aboveground live biomass (g m⁻²) against the mean (±1 SE) cumulative precipitation (mm) at the time of peak biomass measurements. Data are from swale and ridge locations in Control plots from 2005-2014. The shaded data point in the swale data series marks the estimated mean peak total live biomass in 2010.
Figure 2-4. Mean (±1 SE) peak aboveground total (forb + grass) live biomass by topographic position in Control plots from 2005-2014. Within year, asterisks indicate statistical significance between topographic positions at p<0.10*, p<0.05**, and p<0.0001***. Dotted lines indicate the ten-year mean peak aboveground live biomass for swale (grey; 294.6 g m⁻²) and ridge locations (black; 136.3 g m⁻²). Peak total biomass in the swales in 2010 was estimated, and the mean is not from true field values, as the subplot locations were underwater.
Figure 2-5. Mean (±1 SE) aboveground live forb, grass, and total (forb + grass) biomass across the growing season (April-October) in swale and dune ridge locations. Means are from the Control plots, averaged across years from 2005-2014. Within each data series (forb/grass/total), asterisks indicate statistical significance between topographic positions (left and right panel) within each month, at $p<0.10^*$, $p<0.05^{**}$, and $p<0.0001^{***}$. 
Figure 2-6. Mean (±1 SE) peak aboveground live forb, grass, and total (forb + grass) biomass in A) Control B) Grazed and C) Pulse treatments from 2005-2014. The peak biomass values were selected from individual subplots within plots.
Figure 2-7. Mean (±1 SE) aboveground live forb, grass, and total (forb + grass) biomass in A) Control B) Grazed and C) Pulse treatments across the growing season (April-October). Data averaged from 2005-2014.
Figure 2-8. Mean (±1 SE) peak aboveground live biomass of A) grass and B) forb fractions for the Control, Grazed, and Pulse treatment within topographic position (Swale/Ridge), averaged from 2005-2014. Within panels, bars not connected by the same letter are significantly different (p<0.05).
Figure 2-9. Mean (±1 SE) δ\(^{13}\text{C}\) leaf tissue of grass samples from dune ridge locations in the Control and Pulse treatments within early and late growing seasons from 2006-2013. Within seasons, asterisks denote statistical significance at p<0.05. Reference lines indicate the nine-year mean isotopic signature for the Control (solid line; -18.11 \(^{0/00}\)) and Pulse (dashed line; -23.0 \(^{0/00}\)) treatment.
Figure 2-10. Relationship between A) aboveground dead biomass (closed circles) and B) soil erosion (open circles) in the Press treatment from 2005-2014. Data points are means (±1SE) for each sampling date, averaged across plots and topographic locations. Inset in the upper panel is the mean (±1 SE) aboveground dead biomass remaining at the beginning of each growing season (2005-2009). Inset in the lower panel depicts the relationship between the mean (±1SE) remaining amount of aboveground dead biomass (g m⁻²) at the beginning of each growing season and the mean (±1SE) amount of soil erosion (cm).
Figure 2-11. Mean (±1 SE) residence time of aboveground biomass from 2005-2014 for Control, Grazed, and Pulse treatments in A) ridge and B) swale topographic dune locations. Values from 2005, 2008, 2011, and 2014 for the Pulse treatment were omitted, as those were treatment years. Inset displays the mean residence times by treatment within topographic position, where bars not connected by the same letter are significantly different (p<0.05).
CHAPTER 3 - SOIL NITROGEN DYNAMICS IN THE NEBRASKA SANDHILLS: IMPACTS OF CLIMATE VARIABILITY, GRAZING, AND EXPERIMENTAL VEGETATION DISTURBANCES

Abstract

Nitrogen is most often the limiting resource in terrestrial ecosystems and it plays a critical role in maintaining grasslands. Grazing, drought, and shifting plant community composition affect the N cycle by altering the pool of available N (NO$_3^-$–N and NH$_4^+$–N) and the microbial mediated mineralization rate. To understand the role of vegetation disturbances and its effect on the N cycle in the Nebraska Sandhills, I measured KCl extractable soil N (NO$_3^-$–N and NH$_4^+$–N) and conducted in situ soil incubations during the growing season, over 11 years in the Nebraska Sandhills. This research was part of the Grassland Destabilization Experiment (GDEX), which investigated the effects of deferred rotational grazing (Grazed), experimental Pulse vegetation disturbances (one year of vegetation death, two years of recovery), and experimental Press vegetation disturbances (permanent vegetation death). I assessed the 1) general patterns of N availability and supply in undisturbed sand dunes 2) differences in N availability and supply between undisturbed sand dunes and Grazed dunes and 3) the impacts of climate variability and experimental vegetation disturbances. I found that N mineralization and extractable NO$_3^-$–N and NH$_4^+$–N showed strong seasonal cycles, with greater N mineralization and higher soil NH$_4^+$ concentration in swales compared to dune ridges. The estimated annual N mineralization rate for un-grazed Sandhills grasslands is 3.04-
5.13 g N m\(^{-2}\) (mean = 4.18) and the sandy soils contain 0.49-0.55 g N m\(^{-2}\) as ammonium and nitrate (mean=0.525). Cattle grazing had few impacts on the N cycle, only increasing the pool of available NO\(_3^-\) by 70% during one season. Press and Pulse vegetation disturbances produced fluxes of available NO\(_3^-\) (>7.5 µg N g dry soil\(^{-1}\)) and mean N availability was up to 5.5 times greater than Control. Growth of annual species in the Pulse treatment was able to constrain available NO\(_3^-\) in the following recovery years, indicating an ability to maintain ecosystem functioning and provide resilience to perturbations in the N cycle. A severe drought in 2012 produced effects on the N cycle that were similar to the experimental Pulse disturbances, increasing mean available NO\(_3^-\) and NH\(_4^+\) by 390% and 415% during the late growing season and reducing growing season N mineralization rates by 35% compared to non-drought years. These results demonstrate that drought and other vegetation disturbances have a significant ability to alter the N supply and availability in the Sandhills grasslands. In light of climate change predictions for the region, it is likely that these type of disturbances will become more frequent. It is important to understand the effects of these pulse dynamics on the N cycle in this ecologically and economically important ecosystem, and determine the resilience of the Sandhills grasslands to disturbance events.

**Introduction**

After water, nitrogen (N) is most often the limiting resource in terrestrial ecosystems (Chapin III et al. 2011). The availability of N constrains the productivity, decomposition, species diversity, tissue quality, and ability to sequester carbon in terrestrial ecosystems (Wedin and Tilman 1990, Tilman and Wedin 1991a, Reid et al.
These changes in species composition and tissue quality can have positive or negative feedbacks on the N cycle and alter the pool of available N and the mineralization rate of N (Mueller et al. 2013). These two components provide different insights into the N cycle. The pool of N determines what is available for plant uptake, or for loss through atmospheric or groundwater pathways, while mineralization provides an indication of rate of supply of N through microbial processes (Carrillo et al. 2012). The supply of soil N is largely dependent on N mineralization, which is the amount of N in soil released by the microbially mediated conversion of organic N to inorganic forms (NH$_4^+$ and NO$_3^-$). N mineralization is generally measured over a period of 2 to 6 weeks, and is expressed as a rate as mg N kg$^{-1}$ day$^{-1}$ (Robertson et al. 1999). The rate of N mineralization is controlled by soil moisture, temperature, and soil texture, but it is also affected by timing, amount, and quality of above and belowground litter (Burke et al. 1997, Epstein et al. 2002, Jin et al. 2013, Henry and Moise 2014).

Historically, N mineralization represented a direct index of the availability of N for plant uptake. Although recent research has de-emphasized the central role of N mineralization in the cycle, net N mineralization can still be used as an indirect index of N availability (Schimel and Bennett 2004). Additionally, this new paradigm is not necessarily important in the Nebraska Sandhills, as the sandy soils (>94% sand) are well drained, NO$_3^-$ dominated systems, where N mineralization still works as an index of plant available N. This indirect index of N availability can illuminate changes in the N cycle and relate to changes in productivity. The quality of plant material introduced to the soil organic matter dictates the rate of decomposition, immobilization, and mineralization of
Higher quality plant material (low C:N) generally leads to faster decomposition, while low quality plant material (high C:N) leads to slower decomposition as N is limiting (Wedin 2004). Any changes in the plant community composition can affect the litter quality, and subsequently the decomposition, immobilization, and mineralization rates. Thus, altering the characteristics of the plant community can have large effects on the N mineralization rate (Tilman and Wedin 1991b, Díaz and Cabido 2001, Yelenik et al. 2014).

N cycling plays a critical role in maintaining grasslands (Wedin and Tilman 1992). Within the tallgrass prairie, the dominant warm-season C4 grasses are highly N efficient and produce both aboveground (shoots/leaves) and belowground litter (roots) with low tissue N concentrations (Wedin and Tilman 1992). Prairie grasses allocate 60-80% of their annual primary production belowground (Wedin 2004, Lauenroth and Burke 2008), and with the low litter quality (high C:N) decomposition of roots occurs slowly (Bontti et al. 2009, Xu et al. 2012) leading to slower N cycling and a reduction in the soil’s N supply rate. This positive feedback creates an N limited environment that further magnifies the competitive advantage of prairie grasses. Because of this, grasslands are generally characterized by low soil N availability with high nitrification rates (Wedin 1996). Seasonally, the concentrations of inorganic N (NH4+ and NO3−) in grasslands are generally highest in the spring and then reduced over the growing season. To put the differences in the N cycles across grasslands in perspective, the tallgrass prairie (MAP 12.9 °C; MAT 835 mm yr−1) in central North America contains a mean of 0.2-0.4 g N m−2 with net N mineralization rates of 1-4 g N m−2 during the growing season (Knapp et al.
The shortgrass prairie (MAP 8.6 °C; MAT 320 mm yr\(^{-1}\)), located in the western Great Plains, generally have net N mineralization rates of 1.0-2.5 g N m\(^{-2}\) yr\(^{-1}\) and with a mean available N pool of 0.1-0.25 g N m\(^{-2}\) (Lauenroth and Burke 2008). Arid grasslands (MAP 17.2 °C; MAT 298 mm yr\(^{-1}\)) in southwest North America contain a mean available N pool of 0.17-0.41 g N m\(^{-2}\) (Hartley and Schlesinger 2000), while annual net N mineralization (estimated from plant uptake) can reach 5 g N m\(^{-2}\) yr\(^{-1}\) (Havstad et al. 2006). The available pool and supply of N varies across grassland types, but the generally low soil N availability is part of a positive feedback system with the high nutrient-use-efficiency grasses.

Positive feedback systems such as the N cycle in grasslands are susceptible to disturbances (DeAngelis et al. 1989). Heavy grazing can lead to reduced N use efficiency in grasses and a reduction in the amount of biomass allocated belowground (Smith et al. 2014). Additionally, heavy grazing affects the supply rate of N by returning a high proportion of the consumed N back to the system via urine (Augustine et al. 2013). Shifts in precipitation can alter how effective plants and microbes are at acquiring N, increasing the potential for N losses from arid grasslands (Dijkstra et al. 2012). Soil disturbances can also change the soil moisture and temperature, affecting the microbial community, the decomposition of plant materials, and mineralization rates (Evans and Belnap 1999). Because N availability is almost completely regulated by biotic processes, vegetation changes can alter the uptake of N and the rate of decomposition and subsequently the balance between immobilization and net N mineralization (Porazinski et al. 2003). To understand the role of vegetation disturbances in grasslands, one approach
is to consider how these disturbances alter and disrupt the positive feedback between vegetation and N availability (Seastedt 1995).

Vegetation disturbances in the Nebraska Sandhills have the ability to alter plant community composition (Weaver and Albertson 1939). Treatment techniques such as applying “pulse” and “press” disturbances (Bengtsson et al. 2003, Collins et al. 2011) allows researchers to investigate the effects of periodically and permanently removing live vegetation and how it alters the N cycle of the sand dune grasslands. In this research, the Press treatment permanently reduced live vegetation and did not allow re-growth, giving insight into how the N cycle winds down after removing plant uptake. The Pulse treatment has the ability to alter the plant community composition the most, compared to the Control and Grazed treatments. In this study, the Pulse treatment used applications of herbicide glyphosate, which killed all the vegetation in one season, and subsequently large pulses of soil NO₃⁻ occurred in the system as plant litter is decomposed, increasing the potential of leaching losses. This treatment also opened up space for early successional species, which are generally annual, C₃ species with higher tissue quality (lower C:N) compared to the warm-season grasses. The major questions include: Does the vegetation disturbance change the mineralization rates? Is N gradually lost over the Press and Pulse treatment cycles (leaky system), or do the weedy annual species tie up the excess N? How does climate variability affect the processes? Using 11 years of in situ soil incubations, I assessed the 1) general patterns of N availability and supply in undisturbed sand dunes 2) differences in N availability and supply between undisturbed sand dunes and Grazed dunes (the dominant management in the Sandhills) and 3) impacts
of vegetation disturbances (Pulse & Press treatments) on net N mineralization and the availability of extractable \( \text{NO}_3^- \) and \( \text{NH}_4^+ \).

**Methods**

**Study Site & Experimental Design**

This study was conducted within the Grassland Destabilization Experiment, located at UNL’s Barta Brothers Ranch (BBR) in the eastern Nebraska Sandhills. The GDEX project was part of the Sandhills Bio-complexity Project (2003-2008), where the goal of the project was to determine how fluxes of energy, water, and nutrients change as the land cover is altered from continuous grass to bare sand (Wang et al. 2008; Sridhar and Wedin 2009). Mean annual air temperature at BBR is 8.1 °C and mean annual precipitation is 576 mm (Wang et al. 2009a), where 80% of precipitation falls during the growing season between April and September (Schacht et al. 2000). In general, around 90% of the surrounding landscape is composed of upland dunes and dry interdunal areas (swales) dominated by warm-season prairie grasses, while the remaining 10% is subirrigated wet meadows and wetlands. The GDEX site itself is categorized as upland prairie, with no subirrigated wet meadows. The dunes are composed of Holocene eolian sand deposits which overlie Quaternary and/or Pliocene alluvial sand and silt (Wang et al. 2009a). The sand dune soils are mostly composed of Valentine fine sand soils with an average of 94% sand and 0.61% OM at 30 cm depth (Soil Survey Staff et al. 2013). Across the Nebraska Sandhills, the dunes are generally oriented from the northwest to the southeast, and dune heights can be as large as 40m high, 825m long, and 1220m wide.
At BBR, dunes are generally smaller, ranging from 5-20m in height (Wang et al. 2009a) and slopes are 5-15%. Topographic position and aspect across a dune profile affect community composition, with interdunal areas (15% of landscape) having a higher proportion of switchgrass (Panicum virgatum), bluegrass (Poa pratensis), blue grama (Bouteloua gracilis), and sedges (Carex eleocharis). The dune slopes and ridges (85% of landscape) are composed of warm-season, C₄ grasses such as prairie sandreed (Calamovilfa longifolia) and sand bluestem (Andropogon hallii), which are generally located on south facing slopes, while the north slopes contain more cool-season, C₃ grasses such as needle and thread grass (Hesperostipa comata), prairie june grass (Koeleria macrantha), and the shrub leadplant (Amorpha canescens) (Schacht et al. 2000, Milby 2011). Interdunal swale locations also have greater soil carbon content (0.51%) compared to dune slopes and ridges (0.33%) in the top 30cm, with slightly less sand in the top 10cm of swale locations (91.2% sand) compared to dune slopes and ridges (94.4% sand). Interdunal swale A horizons extended to an average depth of 22.8cm (range 6-41cm), while A horizons on the dune ridges extended to an average depth of 11.8cm (range: 6-21cm; Wang et al. 2008).

The GDEX project was set up in 2004 with ten circular plots, 120m in diameter (1.1 hectares total area), which span the profile of one sand dune, from swale to dune top to swale. Within each plot, there were 22 subplots, with varying locations across elevation, aspect, and slope. Each of the ten plots was assigned to one of five management treatments, which range from continuous grass cover to bare sand. Treatments included two Control plots (with no grazing), two Grazed plots (deferred
rotational grazing), two Press plots (no grazing, plant growth prevented with herbicide),
two Pulse plots (no grazing), and two Aggressive plots (no grazing, vegetation killed with
herbicide and disked; treatment not used in this analysis). Press and Pulse treatments
were chosen to examine the response of the Sandhills grassland ecosystem to
experimental vegetation disturbances and provide insights into the stability and resilience
of the sand dune grasslands to permanent and periodic vegetation changes (Bengtsson et
al. 2003, Collins et al. 2011). Plots in the Pulse treatment are on a 3-year schedule, with
vegetation killed with herbicide glyphosate in the first year (reapplications to prevent
regrowth), followed by two years of recovery. Vegetation in the Pulse plots was killed
(without physical disturbance) in 2005, 2008, 2011, and 2014, but allowed to recover
during intervening years (see Chapter 1 for detailed methods). The Press treatment is
similar to the Pulse treatment, but does not allow for vegetation recovery. Vegetation in
the Press treatment plots was killed with herbicide glyphosate in 2005, with further
reapplications as necessary to prevent vegetation regrowth (see Chapter 1 for detailed
methods). The vegetation was kept dead from 2005-2010, at which point the Press
treatment was discontinued because the plots were fully mobilized. Plots in the Grazed
treatment were part of the deferred rotational grazing study at Barta Brother Ranch
(Schacht et al. 2010, 2011). From 2004-2014, the plots in the DRG study pastures were
grazed with 60-132 cow-calf pairs (1.5 AUE) over either three periods (2-14 days) or one
period (30-42 days) during the grazing season (15 May – 15 October). Stocking rates
were moderate, varying from 1.65-2.12 AUM ha$^{-1}$ (see Chapter 1 for more details).
**Sampling Procedure**

Net N mineralization in GDEX was measured over 11 years (2004-2014), with varying numbers of growing season sampling intervals conducted over the course of the project (Table 3-1). Sampling generally occurred over the growing season (April-September) with a single winter sampling interval covering the end of the growing season to the next spring (measured from 2004-2009). In 2010 and 2011, sampling was scaled back to a single monthly sampling interval during the growing season due to time, labor, and resources. From 2012-2014, I expanded the sampling schedule back to the full growing season (Table 3-1). Along with varying sampling intervals, the treatments and subplots within treatments changed over the 11 years. During the 2004 (pre-treatment) growing season sampling period, four subplots in each of the Control and Press treatment plots were measured for net N mineralization. In 2005, six subplots in each of the Control, Pulse, and Press treatment plots were sampled, with the Press sampling ending after 2010, and the Control and Pulse sampling expanding to 12 subplots from 2012-2014. The Grazed treatment was sampled for net N mineralization in six subplots from 2006-2011. Subplot locations were chosen to cover the variation in the topographic range across a sand dune profile.

I estimated net N mineralization monthly by using in situ soil incubations. Soil incubations consisted of using a 1.9 cm diameter plastic tube (PVC), inserted to a depth of 20 cm, and covered with a cap to prevent leaching losses. I sampled initial soil cores concurrently near the placement of the incubation tubes. I extracted ammonium and nitrate from the pre and post incubation soil samples (initial and final) using a 1M KCl
solution, which was shaken for 0.5h and then allowed to settle overnight at 4 °C. The settled solution was then pipetted out and sent to the Ecosystems Analysis Laboratory at the University of Nebraska – Lincoln and analyzed for NH$_4^+$–N and NO$_3^-$–N with a Lachat 8500 Series 2 auto-analyzer. I used a sub-sample from each soil core to determine the gravimetric soil moisture. I calculated net N mineralization (µg N g dry soil$^{-1}$), which is the sum of the net in situ ammonification and nitrification, by summing the final minus the initial values of NH$_4^+$ and NO$_3^-$ for the sampling interval. I calculated growing season and annual net N mineralization by summing the net N mineralization of all sampling intervals within the growing season (April-September) and through the rest of the year.

N availability will be referred to as the concentration of extractable NH$_4^+$–N and NO$_3^-$–N (µg N g dry soil$^{-1}$), using the initial soil samples from a net N mineralization sampling interval (i.e. the concentration of available mineral N). The supply of N is assessed using the net N mineralization during a sampling interval, divided by the number of days in the sampling interval (µg N g dry soil$^{-1}$ day$^{-1}$), and by summing the net N mineralization from sequential sampling intervals (µg N g dry soil$^{-1}$) to obtain the growing season and annual net N mineralization (µg N g dry soil$^{-1}$). I presented values for N availability and supply on a per mass basis, as bulk density was not measured concurrently with soil samples. However, bulk density values from similar Sandhills locations were obtained from the literature (Hellerich 2006), and will be used to transform values to a per mass basis and make general comparisons to other grassland ecosystems.
**Statistical Analysis**

Data was partitioned into four groups to accommodate the variable sampling schedule and analysis goals. The first data set consists of only mineralization data from the undisturbed Control treatment from 2004-2014 (excluding 2010 & 2011 as there was only a single sampling interval). The second data set compares the undisturbed Control and the Grazed treatment during the years that the Grazed treatment was sampled (2006-2009). The third data set consists of the bulk of the mineralization data, comparing the experimental vegetation disturbances (Press & Pulse) to the Control from 2005-2009. The last data set is similar in that it is comparing the Pulse and the Control treatment from 2012-2014. The Press treatment was discontinued after 2010 because the plots had become mobilized, blowing sand dunes, and the years 2010 and 2011 are omitted because there was only a single monthly sampling interval (Table 3-1).

N availability ($\text{NH}_4^+$ and $\text{NO}_3^-$) was analyzed by natural log transforming $\text{NH}_4^+$ and $\text{NO}_3^-$ values during mixed model analysis, and then back-transforming means for graphical presentation. Estimates for standard errors were obtained by multiplying the natural log standard error from model output by the back transformed mean. Daily net N mineralization, N availability (extractable $\text{NH}_4^+$ and $\text{NO}_3^-$), and growing season net N mineralization were analyzed using mixed effects models (JMP Pro v11.0.0) with treatment, topography, season, and year as fixed effects. Topographic position was treated as a categorical variable where the subplots pins in each plot were identified as either ridge or swale locations, depending on their relative elevation within the plot. Random effects included the specific plots and subplot nested within topography and
treatments. Multiple comparisons in mixed model analysis were done using Tukey’s HSD. Model assumptions were checked by predicted-residual plots, Q-Q plots, and scale location plots.

**Results**

*Patterns of N Availability and Supply in Undisturbed Sand Dunes*

Available soil NH$_4^+$ and NO$_3^-$ from the net N mineralization initial soil samples (n=803) were variable in the undisturbed Control treatment over the eleven years of the project (Figure 1-1). The median value for NO$_3^-$ was 0.44 µg N g dry soil$^{-1}$ and 1.06 µg N g dry soil$^{-1}$ for NH$_4^+$. Although 90% of the values for extractable NO$_3^-$ were below 1.64 µg N g dry soil$^{-1}$ and 2.98 µg N g dry soil$^{-1}$ for extractable NH$_4^+$, spatial and temporal variability produced NO$_3^-$ and NH$_4^+$ concentrations as high as 7.80 and 17.50 µg N g dry soil$^{-1}$. The skewed distributions necessitated natural log transforming the extractable soil NH$_4^+$ and NO$_3^-$ values (Figure 3-1 insets) to analyze differences in N availability for treatments, topography, seasons, and years.

Past research in the Sandhills concluded there are differences in vegetation composition between swales, ridges, and north and south facing slopes (Schacht et al. 2000, Stephenson et al. 2013), which may affect organic matter content and nutrient cycling. Partitioning subplots in the undisturbed Control treatment into similar topographic positions yielded significant differences in mean extractable NH$_4^+$ (p=0.0002) and NO$_3^-$ (p=0.0005) from 2004-2014 (Figure 3-2). However, these differences were significant mainly between swale locations and ridges for NH$_4^+$.
(p=0.0003), and swales and slopes for NO$_3^-$ (p=0.0012; p=0.0032). Because of the lack of differences between topographic categories, and to increase statistical power, subplot locations were subsequently divided into ridge and swale locations only.

General patterns of NH$_4^+$ and NO$_3^-$ availability ($\mu$g N g dry soil$^{-1}$) across topography, season, and years were assessed with a mixed model using Control treatment data from 2004-2014 (excluding 2010 and 2011 because there was only one initial soil sample; Table 3-2). Averaging all nine years, mean (± 1 SE) availability of NH$_4^+$ was significantly higher in swale locations (1.29 ± 0.09) compared to ridge locations (0.95 ± 0.06). This topographic difference was not recorded in the availability of NO$_3^-$ (Table 3-2), but there were seasonal differences, where availability of NO$_3^-$ was significantly lower during the early growing season (0.38 ± 0.03) compared to later in the growing season (0.44 ± 0.04). Across the 11 years, there were significant changes in both NH$_4^+$ and NO$_3^-$ availability (Table 3-2). There was a general decline in NO$_3^-$ availability from 2004 to 2009 (0.53 to 0.16), but soil nitrate concentrations increased significantly during the drought in 2012 (1.05 ± 0.11) and then proceeded to decline again (Figure 3-3).

Ammonium availability varied more from 2004 to 2009 (0.69-1.48), but a significant increase, similar to soil nitrate, was recorded in 2012 (2.43 ± 0.17) and decreased in subsequent years (Figure 3-3). In general, mean NH$_4^+$ availability is greater during the early part of the growing season (April-June) compared to the late growing season (July-September) (Figure 3-3). Mean NO$_3^-$ availability was more variable, with greater NO$_3^-$ during the early growing season in some years, but there were significant increases in NO$_3^-$ availability during the late growing season in 2006 and 2007 (Figure
Both NH$_4^+$ and NO$_3^-$ were significantly higher during the late growing season in 2012, increasing by 390% and 415% over the average later growing season NH$_4^+$ and NO$_3^-$ availability. These seasonal differences in NO$_3^-$ availability are generally negatively correlated with soil moisture (Figure 3-4), where higher soil water content in the early growing season (0.08-0.20 mL H$_2$O cm$^{-3}$) is associated with lower NO$_3^-$ availability. Under lower soil moisture availability (<0.08 mL H$_2$O cm$^{-3}$), concentrations of soil NO$_3^-$ increase, with the highest mean NO$_3^-$ availability occurring during the late growing season during the drought in 2012 (Figure 3-4).

Daily net N mineralization ($\mu$g N g dry soil$^{-1}$ day$^{-1}$) during the growing season displayed strong influences of topography and seasonality (Figure 3-5). A two-way ANOVA comparing topography and the incubation period month showed significant effects of month (F-ratio = 14.63 p-value = <0.0001) and topography (F-ratio = 5.08; p-value = 0.0245), with no interactions (F-ratio = 1.25; p-value = 0.2841). From 2004-2014, mean daily net N mineralization ($\pm$SE) was 26% higher in swale locations (0.066 ± 0.006) compared to ridge locations (0.049 ± 0.005). On average, daily net N mineralization increased from April (0.058 ± 0.012) and peaked in June (0.102 ± 0.008), and then subsequently declined through July, August, and September (Figure 3-5).

Because of the seasonality in daily net N mineralization, incubation periods were grouped into early (April-June) and late (July-September) growing seasons (Figure 3-5). As before, a two-way ANOVA comparing topography and season showed significant main effects of topography (F-ratio = 4.55; p-value = 0.0332) and season (F-ratio = 45.41; p-value = <0.0001), where daily net N mineralization was 57% higher in the early
season (0.085 ± 0.005) compared to late season (0.036 ± 0.005). There was also a significant interaction between topography and season (F-ratio = 4.62; p-value = 0.0320). Daily net N mineralization was significantly higher (p=0.0167) in swale locations (0.100 ± 0.008) compared to ridge locations (0.069 ± 0.006) in the early growing season (Figure 3-5). Those differences disappeared during the late growing season, as both swale and ridge locations decreased to a mean daily net N mineralization rate of 0.0361 µg N g dry soil⁻¹ day⁻¹, significantly lower than daily net N mineralization rates for both locations in the early growing season (Figure 3-5). Soil moisture is generally the highest during the early growing season, which is reflected in higher daily net N mineralization rates (Figure 3-4). After the beginning of July, precipitation, and subsequently soil moisture declines, resulting in lower daily net N mineralization rates (Figure 3-5). During the drought of 2012, soil moisture averaged less than 0.05 mL cm⁻³ during the late growing season, and daily net N mineralization rates were slightly negative, indicating net microbial immobilization. Daily net N mineralization rates showed a positive relationship with soil nitrate in ridge locations, during both the early and late growing season (Figure 3-6). However, in the swales, there was no relationship between mineralization and soil nitrate concentrations during the early growing season, and a negative relationship during the late growing season (Figure 3-6).

Growing season, winter, and annual net N mineralization were estimated in the undisturbed Control treatment from 2004-2014 to determine the relative contribution of winter and growing season net N mineralization to annual net N mineralization. Winter and annual net N mineralization estimation was restricted to 2005–2008, as those were
the only years with full winter sampling periods. Net N mineralization (µg N g dry soil\(^{-1}\)) from winter sampling intervals (mean length = 179 days; October-March) contributed on average 13% (2.09 ± SE 0.36) to mean annual net N mineralization (13.72 ± 1.28) in the undisturbed Control treatment from 2005-2008 (Figure 3-7). Over the same period, the relative contribution of winter net N mineralization in the Grazed treatment was similar with 16.27% of the mean annual net N mineralization (15.73 ± 2.13) occurring during the winter period (2.55 ± 0.49). The contribution of winter net N mineralization in the Press (7.30%; 0.73 ± 0.52) and Pulse (12.50%; 2.38 ± 0.29) treatments to annual net N mineralization (Press = 11.51 ± 2.08; Pulse = 12.30 ± 2.58) was lower than Control and Grazed treatments from 2005-2008. Because of the relatively low contribution of winter net N mineralization (7.30%–16.27%) to annual net N mineralization across all treatments, and the variability in winter mineralization sampling, comparisons of net N mineralization across treatments, years, and topography were done with growing season net N mineralization. Though each year has variable growing season sampling lengths, growing season net N mineralization still reflects differences in mineralization rates because the contribution is dominated by late spring and early summer months (Figure 3-5).

From 2004-2014, mean growing season net N mineralization (µg N g dry soil\(^{-1}\)) in the undisturbed Control treatment was 46% higher (F= 9.19; p=0.0030) in swale locations (11.23 ± 0.93) compared to ridge locations (7.41 ± 0.85). There was significant inter-annual variability as well (F=2.22; p=0.0301), where the growing season net N mineralization in 2012 (6.28 ± 1.48) was significantly lower (p=0.0333) than the peak net
N mineralization recorded during the project (14.41 ± 1.97), which occurred in 2007 (Figure 3-7). Although 2012 was not the lowest recorded growing season net N mineralization, it was 35% lower than the average over the other eight years in the study.

**Effects of Grazing**

Differences in the availability of NH$_4^+$ and NO$_3^-$, daily net N mineralization, and growing season net N mineralization were analyzed between the undisturbed Control treatment and the four years of Grazed treatment sampling (2006-2009). Availability of NH$_4^+$ and NO$_3^-$ (from the net N mineralization initial soil samples) was analyzed using two different mixed models to determine effects of treatment, topography, season, and year (Table 3-3, Table 3-4). ANOVA results from the mixed model analyses indicate significant differences in mean available NO$_3^-$ between the Control and Grazed treatment from 2006-2009 (Table 3-3). The Grazed treatment had higher mean (± 1 SE) NO$_3^-$ (0.37 ± 0.04 µg N g dry soil$^{-1}$) compared to the Control (0.22 ± 0.02 µg N g dry soil$^{-1}$). This difference was driven by the increased available NO$_3^-$ in the Grazed treatment at the end of 2007 and beginning of 2008 (Figure 3-8), although not significant enough to produce a treatment × year interaction (Table 3-4). From 2006-2009, there was a decline in both available NO$_3^-$ and NH$_4^+$, similar to results presented earlier on the undisturbed Control treatment (Figure 3-3). Significant differences (Table 3-3) in NO$_3^-$ by season (early season higher) and NH$_4^+$ by topography (swales higher) are comparable to results presented earlier on the undisturbed Control treatment. Adding in Grazed treatment data did produce significant differences in mean NH$_4^+$ availability between seasons with topographic interactions, which was not detected using only the undisturbed Control
treatment data (Table 3-2, Table 3-3). Across the Control and Grazed treatment from 2006-2009, the swale locations did not significantly decrease in mean NH$_4^+$ availability ($\mu$g N g dry soil$^{-1}$) from the early growing season (1.25 ± 0.09) to the late growing season (1.19 ± 0.09). However, ridge locations declined in mean NH$_4^+$ availability from the early growing season (0.94 ± 0.06) to the late growing season (0.71 ± 0.05).

Mixed model analysis of daily net N mineralization ($\mu$g N g dry soil$^{-1}$ day$^{-1}$) comparing treatment, topography, and season resulted in no treatment effects, with only significant differences in topography, season, and the topography × season interaction (Table 3-5). There were no differences in daily net N mineralization between Control and Grazed treatments. The interaction between topography and season was similar to the results presented earlier for the undisturbed Control treatment (Figure 3-5). Swale locations had significantly higher daily net N mineralization in the early season compared to ridge locations, and then there were no significant topographic differences during the late growing season.

Mixed model analysis of the growing season net N mineralization comparing treatment, topography, and year resulted in differences between topography, topography × year, and the treatment × year interactions (Table 3-6); similar to the results presented earlier on the undisturbed Control treatment. There were no main effects of treatment on growing season net N mineralization ($\mu$g N g dry soil$^{-1}$) between Control (11.64 ± 0.96) and Grazed treatments (11.24 ± 0.99) from 2006-2009 (Table 3-6). However, there was a treatment × year interaction where the Grazed treatment had significantly higher
mineralization in 2006, significantly lower in 2007, and then from 2008-2009 there were no differences (Figure 3-9).

**Experimental Vegetation Disturbances**

Data from the Press and Pulse treatments were separated into two groups for analysis. The first group compared available NH$_4^+$ and NO$_3^-$, daily net N mineralization, and growing season net N mineralization between the Control, Press, and Pulse treatments from 2005-2009, because the Press treatments were discontinued after 2010. The second group compared the same responses for the Pulse and the Control treatment from 2012-2014.

From 2005-2009, there were significant differences in available NO$_3^-$ and NH$_4^+$ (µg N g dry soil$^{-1}$) between Control, Pulse, and Press treatments, between seasons, and topographic positions (Table 3-3). Similar to the NH$_4^+$ responses in Control and Grazed treatments from 2006-2009, available NH$_4^+$ (µg N g dry soil$^{-1}$) was higher in swale locations (1.25 ± 0.08) compared to ridge locations (0.96 ± 0.06). There was an overall treatment effect over the five years (Table 3-3), where available NO$_3^-$ (µg N g dry soil$^{-1}$) in the Press treatment (1.44 ± 0.17) was significantly greater than the Pulse treatment (0.88 ± 0.10), which was significantly greater than the Control (0.24 ± 0.03). Treatments also differed in the mean available NO$_3^-$ and NH$_4^+$ between early and late growing seasons (Table 3-3). Across all five years, available NO$_3^-$ was significantly higher later in the growing season relative to the early growing season for each treatment, with 8.5 times more available NO$_3^-$ in the Press treatment, and 3.6 times more available NO$_3^-$ in the Pulse treatment compared to the Control (Figure 3-10).
Available NH$_4^+$ generally declined from the early to late growing season in Control and Pulse treatments, and although not significant, the trend was the opposite in Press treatment (Figure 3-10). Available NO$_3^-$ varied significantly between treatments from 2005-2009 (Table 3-4, Figure 3-8). In the first treatment year (2005), available NO$_3^-$ in the Press and Pulse treatments rose above 7 µg N g dry soil$^{-1}$, about fourteen times the available NO$_3^-$ in the Control (Figure 3-8). From 2005-2009, mean available NO$_3^-$ decreased each year in the Press treatment, but was still significantly higher than the Control in 2009 (Figure 3-11). After herbicide treatment in 2005, the Pulse treatment took two years before mean available NO$_3^-$ was reduced enough that it was not significantly different from the Control (Figure 3-11). The Pulse treatment created significant increases in mean available NO$_3^-$ during treatment years (2005 & 2008; Figure 3-11), although the seasonal peak of NO$_3^-$ availability in 2008 was not as great as in 2005 (Figure 3-8).

From 2012-2014, availability of NO$_3^-$ and NH$_4^+$ (µg N g dry soil$^{-1}$) was significantly different between the Control and Pulse treatment (Table 3-3, Table 3-4). Over the three years, the Pulse treatment had significantly higher available NO$_3^-$ (1.05 ± 0.09) compared to the Control (0.71 ± 0.06), but available NH$_4^+$ was significantly greater in the Control (1.24 ± 0.06) compared to the Pulse treatment (0.99 ± 0.05) (Table 3-3). Averaging across the three years, NH$_4^+$ was significantly higher in the early growing season (1.28 ± 0.07) and reduced in the late growing season (0.95 ± 0.05). Treatments did affect the available NO$_3^-$ between early and late growing seasons (Table 3-4). During the early growing season, available NO$_3^-$ was similar in both Control and Pulse
treatments (0.74 ± 0.07; 0.77 ± 0.08), but in the late growing season, available NO$_3^-$ in the Pulse treatment (1.42 ± 0.14) was significantly higher than the Control (0.69 ± 0.07) values from the early season. Year was a significant effect, where mean NO$_3^-$ and NH$_4^+$ (µg N g dry soil$^{-1}$) across both treatments were significantly higher (1.08 ± 0.08; 1.96 ± 0.11) in the drought year of 2012 compared to the subsequent years in 2013 and 2014 (Table 3-4, Figure 3-8). Mean available NO$_3^-$, but not NH$_4^+$, varied over time between the Control and Pulse treatments (Table 3-4). Both treatments had relatively high mean NO$_3^-$ in the 2012 drought year, and responses were reduced in 2013. However, in 2014 (a treatment year for the Pulse), available NO$_3^-$ increased significantly in the Pulse treatment compared to the Control (Figure 3-8, Figure 3-11).

From 2005-2009, daily net N mineralization (µg N g dry soil$^{-1}$ day$^{-1}$) varied mainly by topographic position and season. However, the treatment effect and the topography × season interaction were approaching significant differences (Table 3-5). As before in the Control and Grazed results, mean (± 1 SE) daily net N mineralization was significantly higher in swale locations (0.070 ± 0.011) than in ridge locations (0.045 ± 0.011). Seasonally, daily net N mineralization was significant between early and late growing seasons (Table 3-5), where, like the undisturbed and Grazed treatment results, mineralization was generally higher in the early growing season (0.065 ± 0.011) than in the later growing season (0.050 ± 0.011). The interaction between topography and season was marginally significant (Table 3-5), but unlike the results from just the Control and Grazed treatment data (Table 3-5), including the Pulse and Press treatment data resulted in no differences in daily net N mineralization between early (0.045 ± 0.012) and
late (0.044 ± 0.012) growing seasons in the ridge locations. Swale locations had significantly greater mean daily net N mineralization in the early season (0.085 ± 0.012), and decreased by 33% to 0.056 ± 0.012 µg N g dry soil⁻¹ day⁻¹ in the late growing season, which was not significantly different from ridge locations in the late growing season.

Treatment effects had a marginally significant impact on mean daily net N mineralization (Table 3-5). From 2005-2009, the Control (0.064 ± 0.012) and Pulse (0.063 ± 0.012) treatment did not differ in the mean daily net N mineralization. However, the mean daily net N mineralization in the Press treatment (0.045 ± 0.012) was reduced at a marginally significant level (p<0.10) in relation to the Control (p=0.0569) and Pulse (p=0.0665) treatments. Over time, mean daily net N mineralization in the Control ranged from 0.046-0.070 µg N g dry soil⁻¹ day⁻¹ from 2005-2009. The Press treatment showed a generally decline from 0.082 µg N g dry soil⁻¹ day⁻¹ in 2005 to 0.028 µg N g dry soil⁻¹ day⁻¹ in 2009. The Pulse treatment was more variable, with lower mean daily net N mineralization in 2006 (0.008 ± 0.014) than both the Control and Press treatment, and higher daily net N mineralization in 2008 (0.125 ± 0.015) than both the Control and Press treatment (Figure 3-12).

Daily net N mineralization (µg N g dry soil⁻¹ day⁻¹) in the Control and Pulse treatment from 2012-2014 did not show any main effects of treatment, or any effects of topography, which were recorded in previous years (2005-2009) using the Control, Pulse, and Press treatment data (Table 3-5). Seasonally, the early growing season was significantly higher (0.093 ± 0.014) than the late growing season (0.033 ± 0.013), with the late season response influenced by the low mineralization during the late growing
season in 2012 (Figure 3-12). From 2012-2014, mean daily net N mineralization was lower during the drought year in 2012 (0.039 ± 0.007) compared to 2013 and 2014 (Figure 3-12).

There were significant differences in growing season net N mineralization (µg N g dry soil\(^{-1}\)) between Control, Press, and Pulse treatments from 2005-2009 and 2012-2014 (Table 3-6). On average from 2005-2009, the Press treatment had significantly lower (p=0.0498) growing season net N mineralization (7.67 ± 0.95) compared to the Control treatment (11.01 ± 0.95). Over the same period, the mean growing season net N mineralization in the Pulse treatment (10.26 ± 0.97) was not significantly different from either the Press or the Control treatment. From 2012-2014, there were no overall treatment differences between the Control and Pulse treatment (Table 3-6).

However, growing season net N mineralization was significantly different between treatments from year to year (Table 3-6). In the first herbicide treatment year (2005) there were no differences in growing season net N mineralization between the treatments, although Press and Pulse treatments had higher mean mineralization (Figure 3-13). Net N mineralization in the Press treatment declined by ~77% after two years, recovered enough in 2008 that it was not significantly different from the Control, and declined again in 2009. Growing season net N mineralization in the Pulse treatment was more variable. In the first recovery year, mineralization was negative, indicating net immobilization (Figure 3-13). This was significantly lower than the Control (p=0.0005) and the Press treatment (p=0.0044), which had decreased by ~46% after the first year. In the second recovery year (2007), mineralization in the Pulse treatment increased by 75%.
from the previous year (Figure 3-13). By 2008 (the second round of herbicide application for the Pulse treatment), mineralization increased by ~55% from the previous year, and was significantly higher than both the Control (p=0.029) and Press treatment (p=0.0012). In 2009, there were no treatment differences between Control, Pulse, and Press. In 2012, there were only marginally significant (p=0.0963) differences between the Pulse and Control treatment, and no differences in 2013. However, in 2014, growing season net N mineralization in the Pulse treatment increased and was significantly different (p=0.0050) from the Control (Figure 3-13).

**Discussion**

The goal of this research was to assess the general patterns of N availability and supply in undisturbed sand dunes, and determine how grazing and vegetation disturbances alter the supply and availability of N. Using 11 years of in situ soil incubations, I determined that climate and inter-annual variability altered N mineralization rates and availability of extractable N. Surprisingly, there were few differences between undisturbed and grazed sand dunes. Implementing experimental vegetation disturbances resulted in significant increases in extractable nitrates and greater variability in growing season net N mineralization. Net N mineralization responses were noisy (Figure 3-12, Figure 3-13), unlike NO$_3^-$ and NH$_4^+$, and would have benefitted from increased replication.
**N Cycling in the Nebraska Sandhills**

I have presented N supply and availability on a per mass basis (µg N g dry soil⁻¹) and not on a per area basis (g N m⁻²) as bulk density was not measured concurrently with soil core sampling. Using an average bulk density of 1.53 g cm⁻³ for the top 20cm of Sandhills soils (Hellerich 2006), the growing season net N mineralization ranges from 1.81-4.41 g N m⁻² (mean = 2.85), while annually (incorporating winter values) net N mineralization ranges from 3.04-5.13 g N m⁻² (mean = 4.18) in the Sandhills. Even though I sampled only the top 20cm of the soil profile, these values should account for a majority of the N cycle, as the top 20cm of soil incorporates the full A horizon at dune ridge areas (mean depth = 11.8 cm) and a majority of the A horizon (mean depth = 22.8 cm) in swale locations (Wang et al. 2008). Additionally, the top 20 cm of soil accounts for 60% and 55% of total soil C and N in a 1m soil profile (see Chapter 5), and contains 60-70% of the total root mass in a 3 m soil core (Wang et al. 2008). These per area basis estimates support CENTURY and other modeling estimates for the Sandhills region, which generally estimate N mineralization at 2.5-6.5 g N m⁻² (Burke et al. 1997, Chapman et al. 2013). Available N (extractable NO₃⁻ and NH₄⁺) varies inter and intra annually, but Sandhills soils generally hold 0.49-0.55 g N m⁻² (mean = 0.52) during the early growing season and 0.50-0.58 g N m⁻² (mean = 0.53) during the late growing season. These estimated values are more similar to the tallgrass prairie in central North America (Available N = 0.2-0.4 g N m⁻²; net N mineralization = 1-4 g N m⁻²; Knapp et al. 1998) than the shortgrass prairie to the west (Available N = 0.1-0.25 g N m⁻²; net N mineralization = 1.0-2.5 g N m⁻² yr⁻¹; Lauenroth and Burke 2008). Although the
Nebraska Sandhills are situated between the short and tallgrass prairie, this suggests that the N cycle in the Sandhills operates in a similar manner to tallgrass prairies.

Daily net N mineralization and the availability of both NO$_3^-$ and NH$_4^+$ displayed strong seasonal patterns and differed based on topographic location. Excluding the drought influenced values, available NO$_3^-$ and NH$_4^+$ fluctuated between 0.06-1.13 and 0.33-1.74 µg N g dry soil$^{-1}$ respectively (Figure 3-8), but spatial and temporal heterogeneity are inherent in this system (Figure 3-1), as in other sand dunes systems (Cain et al. 1999). Swales had higher daily net N mineralization, available NH$_4^+$, and greater total growing season net N mineralization compared to ridge locations. In general, available NH$_4^+$ was higher in the early growing season, and available NO$_3^-$ increased during the late growing season (Figure 3-3). Seasonally, daily net N mineralization starts low (0.02-0.05 µg N g dry soil$^{-1}$ day$^{-1}$) at the beginning of the growing season and increases until it peaks during mid-June (0.07-0.13 µg N g dry soil$^{-1}$ day$^{-1}$), and then subsequently declines through the rest of the growing season (Figure 3-5). These seasonal differences in daily net N mineralization and available NO$_3^-$ are correlated with soil moisture, where higher soil moisture in the early growing season is associated with higher mineralization and lower available NO$_3^-$ (Figure 3-4). As the growing season progresses, soil moisture declines and daily net N mineralization is reduced, while available NO$_3^-$ increases.

However, is the higher mineralization (+46%) in swales driven by the higher soil moisture, or is it also affected by greater soil organic matter and differences in species composition and nitrogen use efficiency? Increased soil organic matter does increase N
mineralization rates in grasslands (Booth et al. 2005), and the swales contain 76% and 68% more soil carbon and nitrogen in the top 10cm (0.81 %C; 0.074 %N) compared to ridge locations (0.46 %C; 0.044 %N) (see Chapter 5). Additionally, species composition can affect N uptake and N use efficiency, along with changing the C:N ratio of litter, which can alter N mineralization (Wedin and Pastor 1993, Wedin and Tilman 1996). In the Sandhills, swales contain a higher proportion of cool-season, C₃ species than ridge locations (Barnes 1986, Volesky et al. 2005, Stephenson et al. 2013), which produce higher quality litter (lower C:N) and adds another variable that affects N mineralization. Similarly, does the higher mineralization in swales lead to higher ambient soil nitrates? The relationship between average daily net N mineralization and available NO₃⁻ indicates a weak or negative relationship in swales, while in ridge locations increased daily net N mineralization is positively associated with increased available NO₃⁻, in both the early and late growing season (Figure 3-6). Extractable soil nitrates concentrations are more variable in the swales locations, especially during the early growing season when increased soil moisture can produce anaerobic environments in the swales. Future research should tease apart the different factors that affect N cycling across topographic locations, and determine whether the difference in N mineralization is reflected in proportionally higher production.

**How Does Drought Affect the N cycle?**

Inter annual differences affect both the pool of available N and the supply rate through mineralization. These differences were especially evident during the drought of 2012, where the mean annual temperature was 1.8 °C higher than the ten year average
and rainfall was 47% (257mm) of the ten year mean (Mallya et al. 2013, Hoerling et al. 2014). The persistent and severe drought in 2012 reduced plant growth and uptake of N, resulting in mean available NO$_3^-$ and NH$_4^+$ that were 390% and 415% higher than average values during the late growing season (Figure 3-3, Figure 3-4, Figure 3-8). During the drought, the peak concentrations of mean available NH$_4^+$ in both the Control and Pulse treatments (5.56 and 4.13 µg N g dry soil$^{-1}$) were some of the highest recorded values over the course of the 11 year project (Figure 3-8). Interestingly, the increases in available NO$_3^-$ are similar in magnitude to experimental Press and Pulse disturbances (Figure 3-8), giving another indication at the severity of the drought in 2012. This dramatic increase in mineral N may also be driven by the reduced activity of soil microbial organisms (Sheik et al. 2011, Hartmann et al. 2013), and similar increases in mineral N in response to droughts have been observed in other grassland ecosystems (Reichmann et al. 2013, Hartmann et al. 2013). The drought also produced lag effects, where the increased pool of available NH$_4^+$ during the late season in 2012 resulted in increased available NO$_3^-$ during the early growing season the following year (Figure 3-3, Figure 3-8) through increased microbial nitrification (Fuchslueger et al. 2014b). The continued high levels of available NO$_3^-$ may be one of the driving factors behind the observed increases in aboveground forb biomass in the 2013 (see Chapter 2).

Additionally, the drought produced significantly reduced soil moisture in the late growing season, which reduced daily net N mineralization enough to produce net immobilization (Figure 3-4, Figure 3-12). A single N mineralization interval in October of 2012 drove the negative values observed in the average seasonal patterns of daily net
N mineralization (Figure 3-5). As daily net N mineralization was reduced in 2012, the growing season net N mineralization decreased by 21% from the nine-year average (Figure 3-7).

During stable, non-drought years, the plant community is able to constrain the availability of N, as extractable NO$_3^-$ and NH$_4^+$ were consistently low (Figure 3-3, Figure 3-8). During the unstable drought year in 2012, the ability of the plant community to constrain the concentrations of extractable NO$_3^-$ and NH$_4^+$ was reduced, resulting in high soil N concentrations and increasing the potential for N loss in the grassland ecosystem (Dijkstra et al. 2012). These results demonstrate that drought has a significant ability to alter N supply and availability in grassland ecosystems as plant and microbial activity is decoupled (Reichmann et al. 2013), and it is likely that drought will play a more important role in altering N cycling than atmospheric CO$_2$ induced changes in the litter C:N ratio (Jin et al. 2013). Climate change predictions for the region indicate that these drought events (with effects similar to our experimental pulse treatment (Figure 3-8, Figure 3-11, Figure 3-13) are likely to occur at a greater frequency (IPCC 2013), and highlights the importance of understanding these pulse dynamics (Collins et al. 2014).

**Impacts of Grazing**

Over the four years of sampling, there were few differences between the Grazed treatment and the undisturbed sand dunes. Growing season net N mineralization in the Grazed treatment differed from the Control in 2006 and 2007, but not in 2008 and 2009 (Figure 3-9), and like the undisturbed sand dunes, it varied between 8-15 µg N g dry soil$^{-1}$ from year to year. The significant increase in extractable NO$_3^-$ from the Grazed
treatment was mainly driven by higher concentrations at the end of 2007 and beginning of 2008 (Figure 3-8), although extractable NO$_3^-$ was generally higher in the Grazed than the Control during the rest of the study. These results support the evidence that cattle can have a significant impact on N-cycling in rangelands (Ritchie et al. 1998, Augustine et al. 2013), and that grazing increases N availability and can act as a buffer against declines in N availability (Anderson et al. 2006). Additionally, there were no effects of grazing on daily net N mineralization (Table 3-5), providing some support to claims that although grazing can accelerate nitrogen cycling in grasslands (Singer and Schoenecker 2003), nitrogen mineralization is not reduced by herbivores on sandy soils (Schrama et al. 2013b, 2013a).

**Experimental Vegetation Disturbances**

Disturbing the aboveground plant community using Press and Pulse treatments altered the availability of extractable NO$_3^-$–N, NH$_4^+$–N, and increased the variability of net N mineralization. Growing season mineralization was generally higher in the Pulse treatment during treatment years, and variable in the recovery years (Figure 3-13), while mineralization in the Press treatment was persistently reduced after the first treatment year. It is interesting to note that even after five years of no plant growth, N mineralization was still occurring at half the rate of Control plots, indicating persistent microbial activity.

Vegetation disturbances resulted in the mean extractable NO$_3^-$ increasing over 5.5 times compared to the Control in the first treatment year in 2005 (Figure 3-8, Figure 3-11). Concentrations of soil nitrates in the Press treatment showed a distinct year-to-year
year decline (Figure 3-8, Figure 3-11), from a peak of 7.56 µg N g dry soil$^{-1}$ in 2005 to 1.35 µg N g dry soil$^{-1}$ in 2009. This gradual decline in extractable soil nitrate indicates the potential for leaching loss in the ecosystem (Chantigny et al. 2013). The Pulse treatment resulted in similar increases in available NO$_3^-$ during treatment years in 2005, 2008, 2011 (data not available), and 2014. However, the plant community displayed a remarkable ability to constrain the concentrations of available NO$_3^-$ during the recovery years, resulting in similar available NO$_3^-$ concentrations after 1-2 years (Figure 3-8, Figure 3-11). Although the plant community composition has changed in the Pulse treatment plots over ten years to a more annual and C$_3$ dominated system (see Chapter 2), the systems shows resilience by maintaining ecosystem functioning and constraining available soil nitrates (Gunderson 2000).

**Conclusions**

Drought, grazing, and vegetation disturbances all had varying effects on components of the N cycle. In the Sandhills, grazing seems to have little effect, only increasing the pool of available NO$_3^-$, which is consistent with results from other grazing studies (Anderson et al. 2006, Schrama et al. 2013b) but the effect is small (+70%) compared to drought or experimental disturbances (+390%). Permanently preventing vegetation growth significantly increases the pool of available NO$_3^-$, although the increased concentration is reduced over time, pointing towards leaching loss from the ecosystem (Chantigny et al. 2013). Pulse cycles of vegetation disturbance produced similar increases in available NO$_3^-$, although these effects are temporary when the system was allowed to recover, demonstrating the resilience of the grassland ecosystem.
Similarly, severe drought acts in much the same way as experimental pulse disturbances of vegetation by producing increased soil nitrate concentrations and reduced mineralization. The risk of leaching to groundwater may be dependent on both spatial and temporal heterogeneity in these disturbance events. Swale locations tend to have greater mineralization and variability in soil nitrate concentrations, while droughts decouple plant and microbial processes and produce large pulses in nitrates. After eleven years the N cycle in Pulse treatments operate in a similar manner to the Control, pointing to resilience in these grasslands to vegetation disturbances and drought.
Table 3-1. Net N mineralization sampling schedule from 2004-2014. Treatments did not start until 2005 and winter sampling was discontinued in 2009.

<table>
<thead>
<tr>
<th>Year</th>
<th>Growing Season Sampling Period</th>
<th>Sampling Intervals within Growing Season</th>
<th>Growing Season Sampling Period (days)</th>
<th>Mean Sampling Interval (days)</th>
<th>Winter Sampling Period</th>
<th>Winter Sampling Interval (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>4/24/04 - 9/26/04</td>
<td>4</td>
<td>155</td>
<td>39</td>
<td>9/26/04 - 4/14/05</td>
<td>200</td>
</tr>
<tr>
<td>2005</td>
<td>4/14/05 - 10/15/05</td>
<td>5</td>
<td>184</td>
<td>37</td>
<td>10/15/05 - 4/6/06</td>
<td>173</td>
</tr>
<tr>
<td>2006</td>
<td>4/6/06 - 10/16/06</td>
<td>6</td>
<td>193</td>
<td>32</td>
<td>10/16/06 - 4/2/07</td>
<td>168</td>
</tr>
<tr>
<td>2007</td>
<td>4/2/07 - 10/10/07</td>
<td>7</td>
<td>191</td>
<td>27</td>
<td>10/10/07 - 4/1/08</td>
<td>173</td>
</tr>
<tr>
<td>2008</td>
<td>4/1/08 - 9/29/08</td>
<td>5</td>
<td>181</td>
<td>36</td>
<td>9/29/08 - 3/30/09</td>
<td>182</td>
</tr>
<tr>
<td>2009</td>
<td>3/30/09 - 9/18/09</td>
<td>5</td>
<td>172</td>
<td>43</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2010</td>
<td>5/17/10 - 6/18/10</td>
<td>1</td>
<td>32</td>
<td>32</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2011</td>
<td>6/23/11 - 7/19/11</td>
<td>1</td>
<td>26</td>
<td>26</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2012</td>
<td>4/20/12 - 10/8/12</td>
<td>5</td>
<td>171</td>
<td>34</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2013</td>
<td>5/6/13 - 10/1/13</td>
<td>5</td>
<td>148</td>
<td>28</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2014</td>
<td>5/9/14 - 10/1/14</td>
<td>5</td>
<td>145</td>
<td>29</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Table 3-2. ANOVA summary of mixed-models for extractable NH$_4^+$–N and NO$_3^-$–N (µg N g dry soil$^{-1}$) for the Control treatment from 2004-2014. Data from 2010 and 2011 were excluded, as there was only one sample point during the year. Bolded numbers indicate significant differences (p<0.05).

<table>
<thead>
<tr>
<th>Source</th>
<th>NO$_3^-$–N</th>
<th>NH$_4^+$–N</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Topo</td>
<td>0.16</td>
<td>0.6917</td>
</tr>
<tr>
<td>Season</td>
<td>6.64</td>
<td>0.0102</td>
</tr>
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<td>Year</td>
<td>40.64</td>
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</tr>
<tr>
<td>Topo × Year</td>
<td>2.81</td>
<td>0.0045</td>
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<tr>
<td>Topo × Season</td>
<td>0.31</td>
<td>0.5797</td>
</tr>
<tr>
<td>Season × Year</td>
<td>28.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Topo × Season × Year</td>
<td>1.56</td>
<td>0.133</td>
</tr>
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</table>
Table 3-3. ANOVA summary of mixed models tested for effects of treatment, topography, and season on extractable NO$_3^-$–N and NH$_4^+$–N (µg N g dry soil$^{-1}$) from initial soil samples of a mineralization sampling period. Separate mixed models were run for responses of NO$_3^-$–N and NH$_4^+$–N to compare the Control vs Grazed treatment (2006-2009), Control vs Pulse (2012-2014), and Control vs Press vs Pulse (2005-2009). Bolded numbers indicate significant differences (p<0.05).

<table>
<thead>
<tr>
<th>Adj. R$^2$</th>
<th>NO$_3^-$–N</th>
<th>NH$_4^+$–N</th>
<th>NO$_3^-$–N</th>
<th>NH$_4^+$–N</th>
<th>NO$_3^-$–N</th>
<th>NH$_4^+$–N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Trt</td>
<td>13.95</td>
<td>0.0013</td>
<td>0.06</td>
<td>0.8148</td>
<td>63.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Topo</td>
<td>21.67</td>
<td>0.0002</td>
<td>0.95</td>
<td>0.3378</td>
<td>8.59</td>
<td>0.0064</td>
</tr>
<tr>
<td>Season</td>
<td>52.49</td>
<td>&lt;0.0001</td>
<td>15.84</td>
<td>&lt;0.0001</td>
<td>121.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt × Topo</td>
<td>2.22</td>
<td>0.1517</td>
<td>0.02</td>
<td>0.8895</td>
<td>2.07</td>
<td>0.144</td>
</tr>
<tr>
<td>Trt × Season</td>
<td>0.03</td>
<td>0.856</td>
<td>0.17</td>
<td>0.6845</td>
<td>13.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Topo × Season</td>
<td>0.13</td>
<td>0.717</td>
<td>0.21</td>
<td>0.6459</td>
<td>0.11</td>
<td>0.7399</td>
</tr>
<tr>
<td>Trt × Topo × Season</td>
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<td>0.8531</td>
<td>0.09</td>
<td>0.9097</td>
<td>1.69</td>
<td>0.1847</td>
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Table 3-4. ANOVA summary of mixed models tested for effects of treatment and year on extractable NO$_3^−$–N and NH$_4^+−$N (µg N g dry soil$^{-1}$) from initial soil samples of a mineralization sampling period. Separate mixed models were run for responses of NO$_3^−$–N and NH$_4^+−$N to compare the Control vs Grazed treatment (2006-2009), Control vs Pulse (2012-2014), and Control vs Press vs Pulse (2005-2009). Bolded numbers indicate significant differences (p<0.05).

<table>
<thead>
<tr>
<th></th>
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</thead>
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<tr>
<td></td>
<td>NO$_3^−$–N</td>
<td>NH$_4^+−$–N</td>
<td>NO$_3^−$–N</td>
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<tr>
<td>Adj. R$^2$</td>
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<tr>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
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<tr>
<td>Trt</td>
<td>12.80</td>
<td>0.0016</td>
<td>0.14</td>
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<td>Year</td>
<td>14.85</td>
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<td>22.44</td>
</tr>
<tr>
<td>Trt × Year</td>
<td>2.03</td>
<td>0.1082</td>
<td>1.18</td>
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Table 3-5. ANOVA summary of mixed models for daily net N mineralization during the growing season (µg N g dry soil⁻¹ day⁻¹). Separate mixed models were run to compare Control vs Pulse (2012-2014; excluding 2010 & 2011), and Control vs Press vs Pulse (2005-2009). Bolded numbers indicate significant differences (p<0.05). Italicized numbers indicate marginally significant differences (p<0.10).

<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td>Adjusted R²</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Trt</td>
<td>0.15</td>
<td>0.22</td>
<td>0.6451</td>
</tr>
<tr>
<td>Topo</td>
<td>17.48</td>
<td>&lt;0.0001</td>
<td>0.4694</td>
</tr>
<tr>
<td>Season</td>
<td>21.68</td>
<td>&lt;0.0001</td>
<td>0.8141</td>
</tr>
<tr>
<td>Trt × Topo</td>
<td>0.54</td>
<td>0.4694</td>
<td>1.02</td>
</tr>
<tr>
<td>Trt × Season</td>
<td>0.06</td>
<td>0.8141</td>
<td>3.82</td>
</tr>
<tr>
<td>Topo × Season</td>
<td>18.48</td>
<td>&lt;0.0001</td>
<td>0.6664</td>
</tr>
<tr>
<td>Trt × Topo × Season</td>
<td>0.19</td>
<td>0.6664</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Table 3-6. ANOVA summary of mixed models for growing season net N mineralization (µg N g dry soil⁻¹). Separate mixed models were run to compare the Control vs Grazed treatment (2006-2009), Control vs Pulse (2012-2014), and Control vs Press vs Pulse (2005-2009). Bolded numbers indicate significant differences (p<0.05).

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Trt</td>
<td>0.08</td>
<td>0.7757</td>
<td>3.37</td>
<td>0.0486</td>
</tr>
<tr>
<td>Topo</td>
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<td>26.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year</td>
<td>0.97</td>
<td>0.4144</td>
<td>8.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt × Topo</td>
<td>1.62</td>
<td>0.2175</td>
<td>2.95</td>
<td>0.0683</td>
</tr>
<tr>
<td>Trt × Year</td>
<td>3.09</td>
<td>0.0337</td>
<td>4.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Topo × Year</td>
<td>5.22</td>
<td>0.0029</td>
<td>1.50</td>
<td>0.2073</td>
</tr>
<tr>
<td>Trt × Topo × Year</td>
<td>1.19</td>
<td>0.3213</td>
<td>1.97</td>
<td>0.0568</td>
</tr>
</tbody>
</table>
Figure 3-1. Histogram and boxplot of extractable soil A) NO$_3^-$ and B) NH$_4^+$ in the undisturbed Control treatment from 2004-2014. Values are from initial soil samples from each net N mineralization sampling interval (n=803). Insets are histograms of natural log transformed NO$_3^-$ and NH$_4^+$ values.
Figure 3-2. Mean (± 1 SE) availability of extractable soil NO$_3^-$–N and NH$_4^+$–N by topographic position across a dune profile. Data are from undisturbed Control treatment from 2004-2014. Data were natural log transformed for analysis and back transformed for presentation. Bars within a data series that are not connected by the same letters are significantly different (p<0.05).
Figure 3-3. Mean (± 1 SE) availability of extractable A) NO$_3^-$–N and B) NH$_4^+$–N (µg N g dry soil$^{-1}$) during the early growing season (April-June), late growing season (July-September), and average availability over the whole growing season (April-September) from 2004 – 2014 in undisturbed sand dunes. Data from 2010 and 2011 are from one sample interval during the early growing season and were not included in mixed model analysis. Within years, bars with asterisk(s) indicate significant (** p<0.05) and marginally significant (* p<0.10) differences between early and late growing seasons. Scatterplot data points for mean growing season availability that are not connected by the same letters are significantly different (p<0.05).
Figure 3-4. Scatterplot of A) Available NO$_3^-$–N (µg N g dry soil$^{-1}$) and B) Daily net N mineralization (µg N g dry soil$^{-1}$ day$^{-1}$) across a soil moisture gradient. Data points are mean (± 1 SE) values over a season (Early and Late) during each year of the study (2004-2014). Nitrate values were natural log transformed to calculate means, and back transformed for presentation.
Figure 3-5. Scatter plot of mean daily net N mineralization (µg N g dry soil\(^{-1}\) day\(^{-1}\)) by topographic position across the growing season in the undisturbed Control treatment (2004-2014). Each point is the mean daily net N mineralization, averaged across incubation periods from 2004-2014. The reference line at July 1 marks the separation of early and late growing seasons in the analysis. Inset is the mean (±SE) daily net N mineralization (µg N g dry soil\(^{-1}\) day\(^{-1}\)) between topographic positions in early (April-June) and late (July-September) growing seasons. Bars not connected by the same letter are statistically significant (p<0.05).
Figure 3-6. Scatter plots of daily net N mineralization versus extractable soil NO$_3^-$–N concentrations by topography (Swale and Ridge) and season (Early and Late growing season) for undisturbed Control sand dunes from 2004-2014 (data points from the late season in 2012 are omitted as they were statistical outliers). Data points are the mean (±1SE) values of each topography × season combination for each year in the study.
Figure 3-7. Mean (± 1 SE) net N mineralization (µg N g dry soil\(^{-1}\)) from 2004-2014 for the undisturbed Control treatment. Bars are dormant season (January-March and October-December), growing season (April-September), and annual net N mineralization. The mean growing season sampling period length was 184 days and the mean winter sampling period length was 179 days. Mean growing season net N mineralization bars that are not connected by the same letters are significantly different (p<0.05).
Figure 3-8. Line plot of geometric mean available A) NO$_3^-$–N and B) NH$_4^+$–N (µg N g dry soil$^{-1}$) for Control, Grazed, Press, and Pulse treatments from 2004-2014. Data points are mean extractable NO$_3^-$–N and NH$_4^+$–N by date, from the initial soil samples of a net N mineralization sampling period. Values were natural log transformed to calculate means, and back transformed for presentation. Pulse treatments (denoted by arrows) were applied in 2005, 2008, 2011, and 2014.
Figure 3-9. Mean (±SE) growing season net N mineralization (µg N g dry soil⁻¹) for the undisturbed Control and Grazed treatments from 2006-2009. Asterisks within a year indicate significant (p<0.05) differences between treatments.
Figure 3-10. Mean (±SE) availability of extractable A) NO$_3^-$–N and B) NH$_4^+$–N (µg N g dry soil$^{-1}$) during the early (April-June) and late (July-September) growing season averaged across Control, Pulse, and Press treatments from 2005-2009. Values were natural log transformed to calculate means and standard errors, and back transformed for presentation. Within panels, bars that are not connected by the same letters are significantly different (p<0.05).
Figure 3-11. Mean (±SE) availability of extractable NO$_3^-$–N (µg N g dry soil$^{-1}$) over the growing season (April-September) for Control, Pulse, and Press treatments. The years 2010 and 2011 are omitted because there was only one sampling interval during June of those years, and the Press treatment was discontinued after 2011. Values were natural log transformed to calculate means and standard errors, and back transformed for presentation. Pulse treatments were applied in 2005, 2008, 2011, and 2014. Extractable NO$_3^-$ by date is presented in Figure 7.
Figure 3-12. Line plot of mean daily net N mineralization ($\mu$g N g dry soil$^{-1}$ day$^{-1}$) for Control, Press, and Pulse treatments from 2005-2014 (excluding 2010 & 2011). Each point is the mean daily net N mineralization for an incubation period, graphed at the midpoint of the period. Pulse treatments (denoted by arrows) were applied in 2005, 2008, 2011, and 2014.
Figure 3-13. Relative growing season net N mineralization (±SE) for Pulse and Press treatments from 2005-2014. The years 2010 and 2011 are omitted because there was only one sampling interval during June of those years. Pulse treatments (denoted by arrows) were applied in 2005, 2008, 2011, and 2014. Bars are the treatment net N mineralization relative to Control (dotted line), and error bars are treatment SE divided by treatment mean, multiplied by the relative treatment net N mineralization.
CHAPTER 4 - SOIL CO₂ RESPIRATION IN THE NEBRASKA SANDHILLS IS RESILIENT TO CLIMATE VARIABILITY AND EXPERIMENTAL VEGETATION DISTURBANCES

Abstract

Soil respiration is the second largest flux of CO₂ from terrestrial ecosystems, and in temperate grasslands, the variability is highly dependent on soil moisture and temperature. However, additional factors such as carbon substrate supply and intra and inter annual variation continue to alter soil CO₂ flux. Climate change impacts are likely to increase temperatures, alter precipitation variability, and affect plant growth and carbon (C) substrate supply. My goals were to 1) assess the differences in soil CO₂ flux when C inputs belowground are reduced or removed and 2) determine the relative importance of soil moisture, soil temperature, soil carbon, topography, and inter and intra annual variation of soil CO₂ flux in the Nebraska Sandhills. To address these goals, I measured soil respiration in Control, Grazed, and experimental vegetation disturbance plots by incorporating Press (permanent vegetation death) and Pulse (one year of vegetation death, two years of recovery) herbicide treatments. I measured soil CO₂ flux every two weeks during the growing season (April-October) from 2005-2014. Topography, intra and inter annual effects are important drivers of soil CO₂ flux. Mean (±1 SE) peak daily CO₂ flux (µmol m⁻² s⁻¹) during the growing season ranged from 1.64 ± 0.05 during the driest year to 2.91 ± 0.07 during the wettest year, with a 10 year mean of 2.26 ± 0.07. Pulse treatments reduced mean growing season soil respiration by 10-40%, and the after six years the Press treatment reduced soil respiration by 76%. Soil CO₂ flux
decreased by 29% during a severe drought in 2012, similar to the effect of the Pulse treatment, which emphasizes the importance of drought in altering ecosystem processes in this grassland ecosystem. The recovery and continued functioning of soil respiration rates after Pulse and drought disturbances demonstrates the resilience of this sand dune grassland ecosystem. These results highlight both the responsiveness and resilience of soil respiration to drought and disturbances, and the dual role (structural vs. functional) of belowground processes in the Sandhills grasslands.

**Introduction**

Soil CO$_2$ flux is an important component of the global carbon cycle and is the second largest flux of CO$_2$ (75-98 Pg C yr$^{-1}$) from terrestrial ecosystems to the atmosphere (Chen et al. 2014, Hashimoto et al. 2015). Semi-arid ecosystems, and temperate grasslands in particular, cover an estimated 7-13% of the global land surface (White et al. 2000) and produce 0.3-0.5 kg m$^{-2}$ yr$^{-1}$ of soil respired CO$_2$ (Mielnick and Dugas 2000, Wang and Fang 2009). CO$_2$ flux from the soil is mainly due to the combined autotrophic and heterotrophic biological activity of roots, bacteria, soil fauna, and fungi, although there are some non-biological sources such as photo-degradation (Rey 2015). Because soil respiration is an important component of the terrestrial carbon cycle, understanding the key controls over spatial and temporal variability of soil CO$_2$ flux provides insight into modeling and predicting soil respiration responses to climate change effects (Vargas et al. 2010, Chen et al. 2014), and can potentially serve as an indicator of state shifts in ecosystems (Potts et al. 2006). This is especially important in semi-arid ecosystems, which dominate the global variability and trend in CO$_2$ uptake and
release (Ahlstrom et al. 2015), and are ecosystems where shifts from one stable state to another can occur through fire suppression, heavy grazing, altered precipitation, and proliferation of invasive species (Bestelmeyer et al. 2003, Bhattachan et al. 2014, Xu et al. 2014, Wonkka et al. 2015).

Variability in soil CO$_2$ flux is driven by several factors, including temperature, precipitation, root biomass, and nutrient and carbon substrate supply. Of these, temperature and precipitation are widely accepted as two of the main factors affecting global variability in soil CO$_2$ flux (Mielnick and Dugas 2000, Raich et al. 2002). In temperate grasslands and semi-arid ecosystems, water availability or more precisely the timing of precipitation (Craine et al. 2012a, Xu et al. 2015) is the dominant factor that regulates rates of soil respiration (Liu et al. 2009, Zhou et al. 2009, Yan et al. 2011). Reduced precipitation and increased variability decrease soil respiration by 8-20% in grasslands ecosystems (Harper et al. 2005, Fay et al. 2008, Thomey et al. 2011). Higher temperatures indirectly increase soil respiration rates in grasslands by stimulating plant productivity and increasing nutrient cycling (Parton et al. 2007). Similar to altered precipitation effects, warmer temperature can increase soil respiration by up to 20%, but these effects are also dependent on soil moisture status (Liu et al. 2002, Zhou et al. 2007) and seasonality (Wayne et al. 2006). The predicted climate change impacts for the region are likely to include increased precipitation variability, higher temperatures, and increased likelihood of drought (IPCC 2013), which will alter soil CO$_2$ flux.

Although soil respiration is sensitive to changes in soil moisture and soil temperature (Harper et al. 2005, Contosta et al. 2011) it is also positively correlated with
root biomass (Schlesinger and Andrews 2000) and substrate availability (Craine et al. 1999). Greater root biomass increases soil respiration rates (Dong et al. 2015), and warming tends to exacerbate the effect (Flanagan et al. 2013). Increased atmospheric CO₂ concentrations also indirectly increase soil respiration through increased substrate supply (Carol Adair et al. 2011). This supply of C belowground is tightly coupled with soil respiration rates, where soil CO₂ flux follows the diurnal pattern of photosynthesis which provides immediate C sources (Bahn et al. 2009). Soil CO₂ flux not only exhibits diurnal patterns, but also seasonal patterns (Dhital et al. 2014). In temperate grasslands, the soil CO₂ flux predictably follows seasonal soil moisture and temperature patterns (Flanagan et al. 2002). Soil respiration also tracks with plant productivity over the growing season, which also influences not only the total soil respiration, but the relative contribution of autotrophic and heterotrophic components (Zhou et al. 2010, Kopittke et al. 2013).

Because soil CO₂ flux is the combination of CO₂ produced by both autotrophs (plant roots) and heterotrophs (microbes/soil fauna) in the soil, it can be viewed as an indicator of belowground production and metabolic activity (Harper et al. 2005, Bowling et al. 2011). If soil CO₂ flux is an indicator of belowground functioning, then in systems where the root-soil complex plays a critical role in ecosystem stability, we can use soil CO₂ flux as a measure of continued ecosystem functioning or a predictor of system collapse. The Nebraska Sandhills, a temperate mixed-grass sand dune grassland, is an ecosystem that relies on the stabilizing effect of vegetation-soil interface to anchor sandy soils against wind erosion. The Sandhills are unlike other semi-arid ecosystems, which
are dominated by shrubs, bunch grasses, and annual weedy species (Havstad et al. 2006, Lauenroth and Burke 2008, Heisler-White et al. 2008). The Sandhills grasslands are composed of dominant, perennial C₄, warm season grasses (Schacht et al. 2000, Mangan et al. 2004). The dunes themselves are stabilized by the grassland root mass, where 60-70% of the root mass occurs in the top 20 cm of soil (Wang et al. 2008) and is recalcitrant to decomposition (Craine and Gelderman 2011). This relatively thin root mass stabilizes the sandy soils, and along with the aboveground vegetation, protects the soil from wind erosion. Any loss of aboveground vegetation, and subsequently the belowground root mass potentially increases the ability of the wind to erode away the soil (Mangan et al. 2004, Li et al. 2007, 2009), creating blowouts and potentially shifting the system into a bare sand, mobilized sand dune state.

Measuring changes in soil CO₂ flux allowed me to assess the effect of vegetation cover, through both experimental vegetation disturbances and by grazing (Owensby et al. 2006). Soil CO₂ flux measures the respiration of plant roots, but more importantly, the respiration of the heterotrophic microbial biomass as it decomposes plant roots and litter. The persistence of the root mass, associated soil heterotrophs, and fungal hyphae, independent of the shoot mass, stabilizes the soil after the ground cover had been disturbed. This will serve to pinpoint at what magnitude of disturbance, and time since disturbance, the below ground microbial functioning is severely reduced (Schmeisser et al. 2009). A decrease in soil CO₂ flux may indicate the loss of the stabilizing root mass due to microbial decomposition, at which point erosion may increase significantly. Additionally, even though soil respiration is highly dependent on soil moisture and
temperature, there are strong intra and inter annual effects that can alter total soil respiration in grassland ecosystems (Flanagan et al. 2002, Xu et al. 2015).

My objectives for this study were to 1) determine the drivers (relative importance of soil moisture, soil temperature, soil carbon, topography, and inter and intra annual variation) of soil CO$_2$ flux in the Nebraska Sandhills and 2) assess the differences in soil CO$_2$ flux when C inputs belowground are reduced or removed by introducing Pulse and Press vegetation disturbances. Specifically, my questions were whether the effects of treatments, topography, season, and year are important because they affect soil temperature and moisture, or are they still significant drivers of soil CO$_2$ flux after accounting for the variation due to soil moisture and soil temperature? Using the experimental vegetation disturbances, how does soil respiration function when removing carbon supply? Can the contributions of autotrophic and heterotrophic soil respiration be untangled? Finally, what is the relative effect on soil CO$_2$ flux of climate disturbances (drought) compared to experimental vegetation disturbances?

**Methods**

**Study Site & Experimental Design**

This study was conducted within the Grassland Destabilization Experiment located at UNL’s Barta Brothers Ranch (BBR) in the eastern Nebraska Sandhills. GDEX project was part of the Sandhills Bio-complexity Project (2003-2008), where the goal of the project was to determine how fluxes of energy, water, and nutrients change as the land cover is altered from continuous grass to bare sand (Wang et al. 2008; Sridhar and
Wedin 2009). Mean annual temperature at BBR is 8.1 °C and mean annual precipitation is 576 mm (Wang et al. 2009a), where 80% of precipitation falls during the growing season between April and September (Schacht et al. 2000). In general, around 90% of the surrounding landscape is composed of upland dunes and dry interdunal areas (swales) dominated by warm-season prairie grasses, while the remaining 10% is subirrigated wet meadows and wetlands. The GDEX site is categorized as upland prairie, with no subirrigated wet meadows. The dunes are composed of Holocene eolian sand deposits which overlie Quaternary and/or Pliocene alluvial sand and silt (Wang et al. 2009a). The sand dune soils are mostly composed of Valentine fine sand soils with an average of 94% sand and 0.61% OM at 30 cm depth (Soil Survey Staff et al. 2013). Across the Nebraska Sandhills, the dunes are generally oriented from the northwest to the southeast, and dune heights can be as large as 40m high, 825m long, and 1220m wide (Bleed and Flowerday 1998). At BBR, dunes are generally smaller, ranging from 5-20m in height (Wang et al. 2009a) and slopes are 5-15%. Topographic position and aspect across a dune profile affect community composition, with interdunal areas (15% of landscape) having a higher proportion of switchgrass (Panicum virgatum), bluegrass (Poa pratensis), blue grama (Bouteloua gracilis), and sedges (Carex eleocharis). The dune slopes and ridges (85% of landscape) are composed of warm-season, C₄ grasses such as prairie sandreed (Calamovilfa longifolia) and sand bluestem (Andropogon hallii), which are generally located on south facing slopes, while the north slopes contain more cool-season, C₃ grasses such as needle and thread grass (Hesperostipa comata), prairie june grass (Koeleria macrantha), and the shrub leadplant (Amorpha canescens) (Schacht et al. 2000,
Interdunal swale locations also have greater soil carbon content (0.51%) compared to dune slopes and ridges (0.33%) in the top 30cm, with slightly less sand in the top 10cm of swale locations (91.2% sand) compared to dune slopes and ridges (94.4% sand). Interdunal swale A horizons extended to an average depth of 22.8cm (range 6-41cm), while A horizons on the dune ridges extended to an average depth of 11.8cm (range: 6-21cm; Wang et al. 2008).

The GDEX project was set up in 2004 with ten circular plots, 120m in diameter (1.1 hectares total area), which span the profile of one sand dune, from swale to dune top to swale. Within each plot, there were 22 subplots, with varying locations across elevation, aspect, and slope. Each of the ten plots was assigned to one of five management treatments, which range from continuous grass cover to bare sand. Treatments included two Control plots (with no grazing), two Grazed plots (deferred rotational grazing), two Press plots (no grazing, plant growth prevented with herbicide), two Pulse plots (no grazing), and two Aggressive plots (no grazing, vegetation killed with herbicide and disked; treatment not used in this analysis). Press and Pulse treatments were chosen to examine the response of the Sandhills grassland ecosystem to experimental vegetation disturbances and provide insights into the stability and resilience of the sand dune grasslands as vegetation is permanently and periodically suppressed (Bengtsson et al. 2003, Collins et al. 2011). Plots in the Pulse treatment are on a 3-year schedule, with vegetation killed with herbicide glyphosate in the first year (reapplications to prevent regrowth), followed by two years of recovery. Vegetation in the Pulse plots was killed (without physical disturbance) in 2005, 2008, 2011, and 2014, but allowed to
recover during intervening years (see Chapter 1 for detailed methods). The Press treatment is similar to the Pulse treatment, but does not allow for vegetation recovery. Vegetation in the Press treatment plots was killed with herbicide glyphosate in 2005, with further reapplications as necessary to prevent vegetation regrowth (see Chapter 1 for detailed methods). Vegetation was kept dead from 2005-2010, at which point the Press treatment was discontinued because the plots were fully mobilized. The Press plots were subsequently planted with native shrubs and grasses beginning in 2011 to facilitate dune stabilization. Plots in the Grazed treatment were part of the deferred rotational grazing study at Barta Brother Ranch (Schacht et al. 2010, 2011). From 2004-2014, the plots in the DRG study pastures were grazed with 60-132 cow-calf pairs (1.5 AUE) over either three periods (2-14 days) or one period (30-42 days) during the grazing season (15 May – 15 October). Stocking rates were moderate, varying from 1.65-2.12 AUM ha\(^{-1}\) (see Chapter 1 for more details).

**Sampling Procedure**

From 2004-2014, the frequency of soil CO\(_2\) flux measurements were variable, but over the 11 years there were at least six sampling dates during the growing season (April-October) in each year. From 2005-2009 sampling generally occurred monthly during the dormant season (November-March) and then every two weeks during the growing season. From 2010-2014, dormant season sampling was discontinued, and growing season sampling varied from every two weeks to once a month. Control, Grazed, and Pulse treatments were sampled continuously from 2005-2014. The Press treatment was sampled from 2005 through 2010, and then was discontinued as the plots had become
mobile sand dunes and efforts began to re-stabilize the soil. The Aggressive treatment is not used in this analysis.

Soil CO$_2$ (µmol m$^{-2}$ s$^{-1}$) flux was measured using the LI-8100 CO$_2$ flux system (LI-COR, Inc., Lincoln, NE). The LI-8100 was equipped with a type-e thermocouple for soil temperature and a Delta-T theta probe for soil moisture. Each sub-plot (n=22 per plot) was equipped with a LI-8100 soil collar, constructed from 4” thin walled PVC. LI-8100 soil collars were placed in the southwest quadrant of the sub-plots until approximately 2 cm remains above the soil surface. Soil CO$_2$ flux measurements were conducted between 0900-1600 hours CST (mean = 1330 hours CST), with values generally reflecting the peak daily soil respiration rate instead of the daily average, which would require continuous flux measurements. When making a measurement with the LI-8100, the thermocouple was inserted into the soil near the collar to a depth of 15 cm and the theta probe was inserted near the collar, but the functional depth was roughly about 7 cm. At each subplot, a single CO$_2$ flux measurement was determined by measuring the ambient CO$_2$ within the IRGA and recording observations every 5 seconds for 60 seconds. The first 20 seconds of observations (4) were discarded due to normalization of the CO$_2$ within the IRGA. The CO$_2$ flux was then calculated by the LI-8100 using the final 8 observations of ambient CO$_2$. The data file containing observations of CO$_2$ flux was checked for integrity using the LI-8100 file viewer software, confirming that fluxes were calculated for each sub-plot and correcting for any errors in calculations. Across the four treatments and ten years of sampling, there were 16,811 observations.
**Statistical Analysis**

Control and Grazed treatment data from 2005-2014 (Grazed treatment was not measured in 2004) were analyzed to determine the relative effects of soil moisture, soil temperature, topography, total % soil C, and intra (early vs late growing season) and inter annual controls over soil CO$_2$ flux. Total percent soil C (0-10cm) was measured in all subplots once during the summer in 2004, 2008, and 2013 (see Chapter 5). Values from the three years were averaged for each subplot and used in the mixed model to account for variation in soil CO$_2$ flux that might not be encapsulated in the topography variable. A multiple regression mixed effects model was used with treatment, year, season, and topography as fixed effects with the specific subplot nested inside the plot as a random effect. Mean total percent soil Carbon, soil moisture, soil temperature, and the interaction of soil moisture and temperature were included as covariates.

Treatment differences were analyzed using two mixed models, with the first model testing the fixed effects of treatment (Control, Grazed, Pulse, Press), year, topography, and growing season from 2005-2010 (Press treatment was discontinued after 2010) while accounting for differences in soil moisture, soil temperature, and the interaction of soil moisture and temperature. The second mixed model addresses the same factors, using Control, Grazed, and Pulse treatment data from 2011-2014.

An additional model analysis was conducted, replacing the treatment and year variables in the previous models with a new variable, number of years dead, which incorporates both time and treatments. This variable assigns a number to plot based on the number of years the vegetation in that plot has experienced the experimental
disturbances. For example, all Control and Grazed treatments are categorized as “0” years dead as they are not subject to experimental vegetation disturbances. The Pulse treatment is categorized as “1” year dead during treatment years (2005, 2008, 2011, 2014) but then a “0” during the subsequent two recovery years. The Press treatment is categorized as “1” year dead in 2005, “2” years dead in 2006, “3” years dead in 2007, and so on. This type of categorization allowed me to combine treatment and inter annual effects to focus on the heterotrophic responses of soil CO$_2$ fluxes. All models were run using JMP Pro (v11). Multiple comparisons in mixed model analysis were done using Tukey’s HSD. Model assumptions were checked by predicted-residual plots, Q-Q plots, and scale location plots.

Because soil moisture, soil temperature, and the interactions between the two are strong predictors of soil CO$_2$ flux, I used the least square means from model output to present statistical differences of categorical variables such as topography, treatments, years, and seasons. These least square means present the relative effects of these variables, independent of the effects of soil moisture and soil temperature, and thus will be different from true means of soil CO$_2$ flux.

**Results**

Variations in soil moisture (Volumetric Water Content, VWC %) and soil temperature (°C), which are strong regulators of soil CO$_2$ respiration, display seasonal patterns in the Nebraska Sandhills. Soil moisture and temperature data from 0-7 cm soil depth were averaged for both Control and Grazed treatments from 2005-2014, with peak soil temperatures (mean = 25.7 ± SE 0.12 °C) during July and into August (Figure 4-1).
Although the variability in soil moisture was greater, the trend is a steady decline during
to the growing season, to a low point in July and into August (6.05 ± 0.12 %). Soil
moisture then begins to recover during the dormant season, increasing from November
through March (Figure 4-1).

Soil CO$_2$ flux ($\mu$mol m$^{-2}$ s$^{-1}$) displayed a similar trend as soil temperature,
increasing during the early growing season from April to the mean peak in June (3.26 ±
0.04), then subsequently declining during the late growing season from July to October
(Figure 4-2). The seasonal trajectories of soil CO$_2$ flux are associated with the seasonal
changes in soil moisture and soil temperature (Figure 4-3). Soil CO$_2$ flux is less than 1
$\mu$mol m$^{-2}$ s$^{-1}$ at the beginning of the growing season in April, but the peak soil CO$_2$ flux in
June is associated with higher soil temperatures and soil moisture (Figure 4-3). After
June, the soil moisture declines rapidly, but soil temperature remains high, and soil CO$_2$
flux subsequently is reduced. Annual soil CO$_2$ flux is dominated by respiration during
the growing season (April-October), and the dormant season respiration (November
through March) accounts for roughly 8% of total annual soil CO$_2$ flux (Figure 4-2 inset).
For this reason, and because a large majority of the measurements were concentrated
during the growing season (89.8%), subsequent analyses used growing season data to
assess intra-annual (early vs late growing season), inter-annual, topographic, and
treatment differences.
Effects of Experimental Vegetation Disturbances on Soil CO₂ Flux

Press Vegetation Disturbances

After controlling for soil moisture, temperature, and their interaction, the Press treatment had a significant effect on mean growing season soil CO₂ flux (Table 4-1), reducing the overall mean flux (0.85 ± 0.05) by ~66% compared to Control (2.53 ± 0.05) and Grazed treatments (2.44 ± 0.05), and by 51% compared to the Pulse treatment (1.74 ± 0.05). Growing season soil CO₂ flux in the Press treatment steadily declined over time. In the first treatment year in 2005, mean growing season soil CO₂ flux in the Press treatment significantly decreased by 30% compared to the Control and Grazed treatments (Figure 4-4). The next year (2006) the soil CO₂ flux was reduced by half from the previous year’s rate, and stood at 40% of the flux relative to the Control (Figure 4-4). Soil CO₂ flux in the Press treatment continued to decline over time, and was significantly lower than all other treatments within each year. However, in 2010, the last year of the Press treatment, the mean growing season soil CO₂ flux almost doubled, from 0.34 to 0.62 µmol m⁻² s⁻¹ (Figure 4-4), although it was still 76.46% less compared to the Control. The relative increase in soil CO₂ flux in 2010 also coincides with increased erosion activity, which accelerated in the winter of 2009-2010 and into the spring of 2010 (Figure 4-5). From 2005-2008, there was an average across all topographic positions of 4.16 ± 0.89 cm of soil eroded in the Press treatment. At the end of the next year in 2009, another 6.39 cm of soil had eroded for total mean of 10.55 ± 2.67 cm of soil erosion. During the winter of 2009 and spring of 2010, erosion accelerated, with an average of
23.17 cm of soil lost during that year, and 33.72 ± 6.10 cm of soil eroded in the Press treatment by the end of 2010 (Figure 4-5).

There were also significant interactions between treatment and the topographic position across a dune and between the early and late growing seasons (Table 4-1). The overall topographic differences, presented earlier, generally held the same relationship in Control, Grazed, and Pulse treatments, but there were not any differences in soil CO₂ flux across topographic locations in the Press treatment (Figure 4-6). Seasonally, there were treatment differences (Table 4-1), where soil CO₂ flux in the Press treatment was reduced by 60% in the early growing season and by 73% in the late growing season, when compared to the Control (Figure 4-6). Additionally, the soil CO₂ flux in the Press treatment was half the rate of the Pulse treatment in the early growing season and only 40% of the rate of the Pulse treatment in the late growing season (Figure 4-6).

**Pulse Vegetation Disturbances**

Like the Press treatment, after incorporating variation due to soil moisture and temperature, there were significant effects of the Pulse treatment during the entire course of the study from 2005-2014 (Table 4-1). From 2005-2010, the overall mean growing season soil CO₂ flux in the Pulse treatment (1.74 ± 0.05) was reduced by ~30% compared to the Control and Grazed treatments (2.52 ± 0.05; 2.44 ± 0.05), and from 2011-2014 the soil CO₂ flux in the Pulse treatment (1.84 ± 0.05) was reduced by 21-24% compared to Grazed (2.33 ± 0.06) and Control (2.41 ± 0.05) treatments (Figure 4-4). As before, these treatment differences varied inter annually (Table 4-1). Mean growing season soil CO₂ flux in the Pulse treatment was significantly reduced in all years from 2005-2014, except
in 2007 and 2011 (Figure 4-4). After three Pulse treatment cycles (herbicide in year one, then two years of recovery), the general trend is a 10-40% reduction in mean growing season soil CO$_2$ flux in treatment years, which carries over into the first recovery year, and then subsequent increases in soil CO$_2$ in the second recovery year (Figure 4-4).

The soil CO$_2$ flux across topographic locations in the Pulse treatment followed the same pattern as the Control and Grazed treatments, although the soil CO$_2$ flux was reduced by 28-38% (Figure 4-6). Like Control and Grazed treatments, the soil CO$_2$ flux was generally highest in swale locations, lowest on the ridges of the sand dunes, and the northeast and southwest slopes had soil CO$_2$ fluxes higher than ridge locations, but lower than swales. The Pulse treatment also varied significantly between early and late growing seasons from 2005-2014 (Table 4-1). Like all other treatments, after incorporating soil moisture and temperature, mean soil CO$_2$ flux was significantly greater in the early season (1.94 ± 0.05) compared to the late season (1.53 ± 0.05) in the Pulse treatment (Figure 4-6). Similar to the topographic responses, soil CO$_2$ flux in the Pulse treatment from 2005-2010 was reduced by 28% in the early season compared to Grazed and Control treatments, and by 30-37% in the late season (Figure 4-6). From 2011-2014, the seasonal relationship of soil CO$_2$ in the Pulse treatment was similar, with significantly greater fluxes in the early season compared to the late season, although the magnitude of change was less than 12% (Figure 4-6). The reduced seasonal differences in the Pulse treatment were similar to the Control, which seasonally did not vary significantly during that interval.
Number of Years Dead

Combining treatment and years into the “years dead” variable in another mixed model resulted in significant effects of soil moisture, soil temperature, and their interaction, similar in contribution as before in previous models (Table 4-2). After accounting for the variation due to soil moisture and soil temperature, there was still a significant interaction between the seasons (early vs late) and the number of years dead, but topography and the topography × years dead interaction were only marginally significant (Table 4-2). The early season soil CO$_2$ flux was significantly higher than late season soil CO$_2$ flux in plots that had not spent any years dead (Figure 4-7). Mean growing season soil CO$_2$ flux averaged 2.26 ± 0.02 µmol m$^{-2}$ s$^{-1}$ in plots with live vegetation, and over six years, the growing season soil CO$_2$ flux exponentially declined to 0.57 ± 0.10 µmol m$^{-2}$ s$^{-1}$ (Figure 4-7 inset).

Soil CO$_2$ flux in the early season in plots experiencing their first dead year were not significantly different from late season soil CO$_2$ flux from the previous year (Figure 4-7), but then there was a significant decrease in soil CO$_2$ flux during the late season of the first dead year. This difference was likely from the variable application of herbicide treatment in Pulse treatment years, which may allow for higher soil CO$_2$ flux until the treatment was applied. Soil CO$_2$ generally declined in the following years, but there was a 43% increase in soil CO$_2$ flux in the early season of the sixth dead year compared to the early season in the fifth dead year (Figure 4-7). The soil CO$_2$ flux then dropped by 86% from the early to the late season in the sixth dead year (Figure 4-7). This significant increase and subsequent decrease followed widespread dune mobilization (erosion) in the
Press treatment, which occurred during the winter of 2009 and spring of 2010 (Figure 4-5). This increase in erosion corresponds to the time after the late season of the fifth dead year and early season of the sixth dead year (Figure 4-7), although these effects may be driven by specific subplot locations or time periods.

**Controls over Soil Respiration in Undisturbed Sand Dunes**

Mixed-model analysis of soil CO$_2$ flux ($\mu$mol m$^{-2}$ s$^{-1}$) for the Control and Grazed treatments from 2005-2014 revealed significant effects of topographic position, season, year, soil moisture, soil temperature, and mean % total soil carbon (Table 4-3). However, the effect of grazing on soil CO$_2$ flux was not significant (Table 4-3). Although interactions between predictor variables were significant, they were not inherently interesting, and only the main effects are presented. After incorporating the effects of various factors (soil carbon, soil moisture, soil temperature, and moisture $\times$ temperature), there were significant effects of topography, intra annual, and inter annual variability (Table 4-3). Predicted mean (±1 SE) soil CO$_2$ flux was correlated with observed measurements (Figure 4-8). When observed and model predicted values were averaged by sampling date, linear regression shows that the model predicts soil CO$_2$ flux well across the range of observed values (Adj. $R^2 = 0.78$; Figure 4-8 inset), except in ranges where soil CO$_2$ flux is <1 $\mu$mol m$^{-2}$ s$^{-1}$. The model predicted the mean (±1 SE) seasonal fluxes well from 2005-2014, with over predictions in the late season in 2005 and early season in 2007, and under predictions in the late season of 2009 (Figure 4-8).

Intra-annually, mean soil CO$_2$ flux ($\mu$mol m$^{-2}$ s$^{-1}$) is generally lower in the late growing season (2.28 ± 0.02) compared to the early (2.59 ± 0.02) growing season (Figure
4-9). Across topographic positions of a sand dune, mean (±1 SE) soil CO$_2$ flux is lowest in ridge locations (2.17 ± 0.04), which were significantly lower than slope (northeast = 2.48 ± 0.03; southwest = 2.57 ± 0.03) and swale locations (2.53 ± 0.05). However, swale locations are not significantly different (p=0.7998; p=0.8816) than the northeast and southwest slope locations (Figure 4-9). Inter-annually, mean annual soil CO$_2$ flux fluctuated from 2.14-2.82 µmol m$^{-2}$ s$^{-1}$ in non-drought years. Mean soil CO$_2$ flux was significantly lower in 2012 (1.78 ± 0.05) compared to all other years in the study (Figure 4-9). As expected, there is a positive linear relationship between growing season precipitation and mean growing season soil CO$_2$ flux (Figure 4-9). The highest annual precipitation during the study occurred in 2008 with 700 mm of precipitation, with 93% (652 mm) falling during the growing season. Conversely, the lowest annual precipitation (257 mm) occurred during the drought of 2012, where 193mm of precipitation fell during the growing season, which was less than 40% of the average growing season precipitation from 2004-2014. During average and above average precipitation years, mean growing season soil CO$_2$ flux ranges from 2.14-2.82 µmol m$^{-2}$ s$^{-1}$, with little increase in wet years (Figure 4-9). However, soil CO$_2$ flux seems to respond more in drought years (2012) than in years with excess precipitation such as in 2008 (Figure 4-9), where soil CO$_2$ flux decreased to 1.78 ± 0.05 µmol m$^{-2}$ s$^{-1}$, which was 30% less than the mean growing season soil CO$_2$ flux in non-drought years.
**Discussion**

In the Nebraska Sandhills, as in most other grassland ecosystems, soil moisture and soil temperature strongly regulate soil respiration rates (Mielnick and Dugas 2000, Harper et al. 2005, Zhou et al. 2009, Thomey et al. 2011). In the Sandhills (>90% sand), water infiltrates quickly (Billesbach and Arkebauer 2012) and the top 7cm of soil has low volumetric water content (median = 6.80%). Measured soil CO$_2$ flux seems to be more dependent on soil temperature than the soil moisture, which is chronically low. The relationship of predictive power for soil CO$_2$ flux between soil moisture and soil temperature may change as soil depth increases (De Graaff et al. 2014). However, the factors that have strong associations with soil moisture and soil temperature (such as topography, intra-annual variation, or inter-annual variation) still produced significant effects on soil CO$_2$ flux. Intra-annual variability (early vs late growing season) was still a significant predictor of soil CO$_2$ flux, demonstrating the tight linkage of carbon supply via vegetation growth on soil respiration rates (Table 4-3). Clearly, soil moisture and soil temperature are the main drivers of soil respiration (Chen et al. 2010), but additional factors such as topography, soil carbon availability, intra-annual variation, or inter-annual variation are still important in explaining soil respiration responses.

A simple mixed-model containing soil moisture, soil temperature, percent soil carbon, topography, season, and year performed reasonably well in predicting soil CO$_2$ flux in this grassland ecosystem (Table 4-3, Figure 4-8). From 2005-2014, the mean daily peak soil CO$_2$ during the growing season was 2.26 ± 0.07 µmol m$^{-2}$ s$^{-1}$, with observed values ranging from 1.64 ± 0.05 µmol m$^{-2}$ s$^{-1}$ in drought years to 2.91 ± 0.07
µmol m$^{-2}$ s$^{-1}$ in wet years. However, the model tended to under-predict soil respiration at low values (<1 µmol m$^{-2}$ s$^{-1}$), indicating that other factors such as C substrate availability (Bahn et al. 2009) are important in regulating soil CO$_2$ flux when moisture and temperature aren’t limiting (Xu et al. 2004). Unexpectedly, there were no significant differences between mean growing season soil CO$_2$ flux in the Grazed treatment and the Control (Table 4-3). Grazing has been shown to reduce grassland soil respiration by as much as 50% due to reduced root mass (Johnson and Matchett 2001) but grazing effects in grasslands can be highly constrained by moisture limitations during dry periods (Hubbard 2003). Additionally, because the grazing effect was moderate (Stephenson et al. 2013, 2015) and short term (mean = 36 days), the effect may have been washed out as the data was averaged in the model. Before the study started in 2004, all plots were grazed, but after ten years of release from grazing pressure, there is no evidence of divergence in the mean daily peak soil respiration rates during the growing season between Grazed and Control treatments (Figure 4-4).

Topographically, mean soil CO$_2$ flux between swales and sand dune slopes were not significantly different, but soil respiration rates on dune ridges remained significantly lower (Figure 4-9). Total soil C on dune ridges is about 75% less than the total soil C content in swales (see Chapter 5; Wang et al. 2008), but this effect was accounted for in the model. This suggests that species composition differences between ridge and swale locations, with a greater proportion of cool-season C$_3$ species in swale locations (Stephenson et al. 2013) potentially affected mean soil respiration rates (Metcalfe et al. 2011). Seasonally, early season soil respiration is significantly higher than the late
season (Figure 4-9). This highlights both the importance of autotrophic respiration in the early season compared to the late season when grasses senesce (Xu et al. 2004) and the greater photosynthetic capacity and translocation of C substrates belowground in the early growing season (Bremer and Ham 2002).

**Belowground Controls on Sand Dune Stability**

In this study, Press and Pulse disturbances on the grassland vegetation had significant negative impacts on soil CO$_2$ fluxes. Treatments affected soil moisture and temperature by reducing water loss through plant uptake and increasing the fraction of solar radiation that reaches the soil. However, even though treatments affected soil moisture and temperature, the effects were still significant after incorporating the soil moisture and soil temperature differences in the mixed-model. These treatments allowed me to study the effects of removing autotrophs permanently (Press) and temporarily (Pulse), and determine how soil respiration responds when it is dependent on C substrates from previous years. When autotrophs are permanently removed, soil CO$_2$ flux declined as root activity decreased (Craine et al. 1999) and available C was decomposed (Shi et al. 2014). After six years of zero vegetation growth, soil CO$_2$ flux declined to 20% of Control values, with no topographic differences. This treatment allowed me to untangle the autotrophic and heterotrophic components of soil respiration. As in the undisturbed sand dunes, there were still seasonal differences in soil respiration, even with the removal of autotrophic respiration components (Figure 4-6, Figure 4-7). Soil CO$_2$ flux was higher in the early season compared to the late season, potentially indicating that the early season is better for heterotrophs (Zhou et al. 2007, Gomez-Casanovas et al. 2012), with
higher moisture availability and lower temperatures. The larger decline in soil respiration in the late growing season in the Press treatment could be due to the rapid decomposition of what little available C is left (Hamerlynck et al. 2013).

On the other hand, the soil CO₂ flux responses in the Pulse treatment give us insight into belowground resilience to reduced C inputs, and the ability to recover after aboveground vegetation disturbances. The Pulse treatment effectively reduced mean growing season soil CO₂ flux by 10-40% (Figure 4-4). Unlike the Press treatment, soil CO₂ flux differed topographically with greater soil respiration in swale locations compared to ridges, similar to Control and Grazed treatments, although the overall mean fluxes were reduced (Figure 4-6). The year after a Pulse treatment event, the soil respiration declined by another 5-20%. This is likely due to removal of perennial plants and the delayed colonization of annual weedy species in the following year, which provide the available C substrates for respiration. However, in the second year after a pulse event, soil respiration recovered by 15-19% (Figure 4-4). Over the ten years of the study, the mean growing season soil CO₂ flux never recovered to Control levels. These results show that soil respiration and belowground processes are highly responsive to regular pulse disturbances (Harper et al. 2005), but they are also resilient and continue to function at reduced levels (Fry et al. 2014), even after ten years. Although the belowground functioning displays resilience to these disturbances, the Pulse events have altered the plant community composition (see Chapter 2) and may potentially be altering microbial and fungal communities (Fuchslueger et al. 2014a).
Soil CO$_2$ flux exponentially declined as the number of years of vegetation death increased (Figure 4-7), but there was a significant increase in the flux rate in the early growing season of the sixth dead year compared to the late growing season of the fifth dead year. The soil CO$_2$ flux declined by 86% from the early to the late season in the sixth dead year (Figure 4-7). I did not find any evidence of specific dates within a season, or individuals plot and subplots, which could have driven the increased flux in the early season of the sixth dead year. The few studies that address the impact of erosion on soil CO$_2$ fluxes conclude that effect of erosion is indirect through the impacts on soil moisture and temperature (Bajracharya et al. 2000a, 2000b). However, even after my model accounted for the effects of soil moisture, soil temperature, and their interaction, significant intra and inter annual effects were still significant (Table 4-2). Although this study was not set up to determine mechanistic causation, the timing of increased erosion and variable soil CO$_2$ flux are related (Figure 4-5). A potential explanation could be increased photodegradation as erosion exposes carbon sources in the soil profile (Rey et al. 2011, Rey 2015), along with increased heterotrophic respiration due to these newly exposed carbon sources which are consumed rapidly as the A horizon (range 6-21cm; Wang et al. 2008) erodes away.

Categorizing the treatments and years into a “years dead” variable also allowed me to estimate the relative contribution of autotrophic and heterotrophic respiration to total soil respiration. I assumed that plots that spent a year under vegetation suppression (Press 2005, Pulse 2005, 2008, 2011, and 2014) were dominated almost entirely by heterotrophic respiration, as the autotrophic component was removed. Dividing the mean
soil CO₂ flux of one year dead plots (1.83 µmol m⁻² s⁻¹) by plots without vegetation suppression (2.26 µmol m⁻² s⁻¹) shows that the potential contribution of heterotrophic respiration to mean growing season soil CO₂ flux is 81.05%. Additionally, the relative contribution can be broken down by season (Figure 4-7) where heterotrophic respiration potentially contributes an estimated 92.5% in the early growing season, but only 68.79% in the late growing season. These estimates are likely high as partitioning source components of soil CO₂ flux was not the focus of our study, and recently dead roots most likely primed heterotrophic respiration (Kuzyakov 2006, 2010, Xiao et al. 2015). Some autotrophic respiration was probably included in Pulse treatment plots as herbicide treatments were delayed in some years, especially in 2011 (Figure 4-4), allowing for early season weedy annuals to germinate and grow. In other grassland systems, heterotrophic respiration accounts for 90% of dormant season respiration, only 35% during summer months, and 65% of the overall annual soil respiration (Zhou et al. 2007). It seems in the Nebraska Sandhills mixed-grass ecosystem, heterotrophs dominate soil respiration in the early growing season, but in the late growing season the contribution of autotrophic respiration increases.

**Relative Impact of Drought on Soil CO₂ Flux**

Soil CO₂ flux responds to both climate variability (drought) and experimental vegetation disturbances, but what is the relative effect of each? In 2012, the Nebraska Sandhills experienced exceptional drought for a majority of the growing season, and it was among the most severe in the last 117 years for the region. During the growing season, daily mean temperatures increased by +1.9 – 6.1 °C and annual precipitation
was 47% (257mm) of the ten year average (Mallya et al. 2013, Hoerling et al. 2014). This extreme climatic disturbance reduced growing season mean soil CO$_2$ flux in Control and Grazed treatments to 1.78 µmol m$^{-2}$ s$^{-1}$, which was a 29% reduction compared to mean soil CO$_2$ flux in non-drought years (Figure 4-8, Figure 4-9). The effect of experimental vegetation disturbances are similar to the drought disturbance effect. Averaging the mean growing season soil CO$_2$ flux of plots in their first dead year (Press 2005, Pulse 2005, 2008, 2011, 2014) there was a 28.5% reduction (1.82 µmol m$^{-2}$ s$^{-1}$) in soil CO$_2$ flux compared to non-drought years (Figure 4-4, Figure 4-7). Other studies have shown similar impacts, with simulated droughts producing up to 50% reductions in soil respiration (Selsted et al. 2012, Hamerlynck et al. 2013). Droughts in this region are likely to increase in frequency, intensity, and duration (Kunkel et al. 2013), although permanent Dust-Bowl like conditions are unlikely (Hoerling et al. 2012). In light of these projected impacts, I have shown that drought effects can match those of physically preventing vegetation growth, and it is important to understand the immediate soil respiration responses, as well as the legacy impact of drought events (Wang et al. 2013).

**Estimated Annual Soil C Flux**

Although mass balancing the C budget for the Sandhills ecosystem was not my objective, I estimated the annual C respired in this grassland system using both the fitted regression equation for the average soil CO$_2$ flux (Control and Grazed treatments from 2005-2014) by day of year (Figure 4-2), and by the mean soil CO$_2$ flux for each month (Figure 4-2 inset). At the GDEX study site in the Nebraska Sandhills, the estimated annual soil C respiration is 487 g C m$^{-2}$, while using the mean monthly fluxes resulted in
495 g C m\(^{-2}\) yr\(^{-1}\), which are both similar to the estimated annual soil CO\(_2\) flux of 389 – 442 C m\(^{-2}\) yr\(^{-1}\) in temperate grasslands (Raich and Schlesinger 1992, Wang and Fang 2009). My estimates assumed static soil respiration rates within days and months, and thus most likely overestimated the annual soil C respired. This overestimation is because sampling was conducted during the middle of the day (mean = 1330 CST) when photosynthesis levels peak, and plant-derived substrates provide an important, and tightly coupled, C source for soil respiration (Bahn et al. 2009). I did have continuous soil CO\(_2\) flux data using a LI-8100 Automated system, at a single Control plot location in 2006, where soil respiration was sampled every 30 minutes from May 17 to October 16 (Wedin, unpublished data). To determine the overestimation of these annual soil CO\(_2\) flux values, I regressed the daily maximum soil CO\(_2\) flux by the daily mean soil CO\(_2\) flux (R\(^2\) = 0.92; Daily Max = 1.3044 × Daily Mean + 0.1440). Using the slope of the regression, the annual estimates of soil CO\(_2\) flux are roughly 30% too high, and are more likely to be 340-347 g C m\(^{-2}\) yr\(^{-1}\). Even with a 30% overestimation, my values still fit within the spectrum of annual soil respiration in other Great Plains grasslands, including the Kansas tallgrass prairie (858–1072 g C m\(^{-2}\) yr\(^{-1}\); Bremer and Ham 2002, Smith and Johnson 2004), southern tallgrass prairies (662–1004 g C m\(^{-2}\) yr\(^{-1}\); Dugas et al. 1999, Mielnick and Dugas 2000, Zhou et al. 2006), shortgrass prairies (230 g C m\(^{-2}\) yr\(^{-1}\); Coleman 1973), and northern mixed-grass prairies (220.91 g C m\(^{-2}\) yr\(^{-1}\); Frank 2002).
Conclusion

Soil respiration in grassland ecosystems are highly sensitive to variability in soil moisture and soil temperature, yet factors such as topography and intra and inter annual variation are still key, highlighting the importance of species composition, seasonal C substrate availability, and inter-annual controls over soil CO₂ flux. In the Nebraska Sandhills, drought is a significant ecological disturbance, reducing soil respiration rates as effectively as our experimental Pulse herbicide treatments. The recovery and continued functioning of soil respiration rates after Pulse and drought disturbances indicates the resiliency of this sand dune grassland ecosystem. Permanently preventing vegetation growth (Press disturbance) resulted in soil respiration declining exponentially as heterotrophic components (fungi and bacteria) consumed the remaining available C. After five years, the belowground activity (soil respiration) persisted but declined significantly, indicating the loss of roots and associated connective agents (fungal hyphae, bacteria, soil fauna) as a structural component in the soil, which correlated with increased erosion rates. These results highlight both the responsiveness and resiliency of soil respiration to drought and disturbances, and the dual role (structural vs. functional) of belowground processes in the Sandhills grasslands.
**Figures & Tables**

Table 4-1. Mixed-model ANOVA table effects on soil CO$_2$ flux (µmol m$^2$ s$^{-1}$) in Control, Grazed, Pulse, and Press treatment plots from 2005-2010 (Press treatment was discontinued after 2010) and Control, Grazed, and Pulse treatment plots from 2011-2014. Bolded numbers indicate significant differences (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted R$^2$</td>
<td>0.65</td>
<td>0.60</td>
</tr>
<tr>
<td>Source</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Year</td>
<td>37.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>271.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Topography</td>
<td>13.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>280.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year × Treatment</td>
<td>42.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year × Topography</td>
<td>2.48</td>
<td>0.0012</td>
</tr>
<tr>
<td>Year × Season</td>
<td>47.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment × Topography</td>
<td>2.05</td>
<td>0.0377</td>
</tr>
<tr>
<td>Treatment × Season</td>
<td>3.21</td>
<td>0.0221</td>
</tr>
<tr>
<td>Topography × Season</td>
<td>0.23</td>
<td>0.8736</td>
</tr>
<tr>
<td>Soil Temperature (°C)</td>
<td>3824.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Moisture (%)</td>
<td>2154.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Moisture × Soil Temperature</td>
<td>1214.13</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 4-2. Mixed-model ANOVA table comparing effects on soil CO$_2$ flux from 2005-2014. Treatment and time (years) were condensed into the “Years Dead” variable. Bolded numbers indicate significant differences (p<0.05). Italicized numbers indicate marginally significant differences (p<0.10).

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>d.f. Den</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topography (NE/Ridge/SW/Swale)</td>
<td>3</td>
<td>1487</td>
<td>2.22</td>
<td>0.0838</td>
</tr>
<tr>
<td>Season (Early/Late)</td>
<td>1</td>
<td>8653</td>
<td><strong>102.81</strong></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Years Dead</td>
<td>6</td>
<td>1502</td>
<td><strong>224.87</strong></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Topography × Season</td>
<td>3</td>
<td>8880</td>
<td>0.38</td>
<td>0.7643</td>
</tr>
<tr>
<td>Topography × Years Dead</td>
<td>18</td>
<td>1470</td>
<td>1.52</td>
<td>0.0757</td>
</tr>
<tr>
<td>Season × Years Dead</td>
<td>6</td>
<td>8613</td>
<td><strong>23.37</strong></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Moisture (%)</td>
<td>1</td>
<td>9205</td>
<td><strong>3997.92</strong></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Temperature (°C)</td>
<td>1</td>
<td>9249</td>
<td><strong>4911.56</strong></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Moisture × Soil Temperature</td>
<td>1</td>
<td>9789</td>
<td><strong>1304.36</strong></td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Adj. $R^2 = 0.65$  
Obs: 9997
Table 4-3. Mixed-model ANOVA table comparing effects on soil CO$_2$ flux in Control and Grazed treatment plots from 2005-2014.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>d.f. Den</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>810.8</td>
<td>2.22</td>
<td>0.1364</td>
</tr>
<tr>
<td>Topography</td>
<td>3</td>
<td>751.1</td>
<td>23.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>4714</td>
<td>114.94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year</td>
<td>9</td>
<td>859</td>
<td>38.75</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Carbon (%)</td>
<td>1</td>
<td>785.1</td>
<td>55.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Temperature (°C)</td>
<td>1</td>
<td>4858</td>
<td>4050.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Moisture (%)</td>
<td>1</td>
<td>5224</td>
<td>2505.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil Temperature × Soil Moisture</td>
<td>1</td>
<td>5220</td>
<td>1026.31</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Adj. $R^2 = 0.60$    Obs: 5248
Figure 4-1. Mean (±1 SE) soil temperature (°C) from 0-10cm and mean (±1 SE) soil moisture from 0-10cm (volumetric water content %) by day of year using Control and Grazed treatment data from 2004-2014.
Figure 4-2. Mean (±1 SE) daily peak soil CO₂ flux (µmol m⁻² s⁻¹) by day of year using Control and Grazed treatment data from 2004-2014. Data points were fit with a three-parameter peak Gaussian curve (±95% CI) (SigmaPlot v13.0). Equation coefficients are \( a = 3.163, b = 595.367, X₀ = 191.247 \). Vertical lines denote the beginning of the growing season (April 1), the division between the early and late growing season (July 1), and the end of the growing season (November 1). Inset is the mean (±1 SE) daily peak soil CO₂ flux by month, using the same Control and Grazed treatment data from 2004-2014.
Figure 4-3. Seasonal trajectories of mean daily peak soil CO$_2$ flux for each month across A) volumetric water content and B) soil temperature. Data points are means (±1 SE) of Control and Grazed treatment data from 2005-2014. The first point in the seasonal sequence is the monthly mean for April, then May, June, July, August, September, and October.
Figure 4-4. Annual soil CO\textsubscript{2} flux for Grazed, Pulse, and Press treatments relative to the Control from 2005-2014. The Press treatment was discontinued after 2010. Data points are the treatment least square mean growing season soil CO\textsubscript{2} flux relative to Control (horizontal line at 1.0), and error bars are treatment standard error divided by treatment mean, multiplied by the relative treatment soil CO\textsubscript{2} flux. Arrows denote herbicide application years in the Pulse treatment.
Figure 4-5. Scatterplot of mean (±1 SE) A) soil CO₂ flux and B) soil lost from original (2004) soil height for each sampling date, averaged across all topographic positions, in the Press treatment. The arrow denotes when the Press treatment started in 2005. Inset is the relationship between mean (±1 SE) growing season soil CO₂ flux and mean annual erosion from 2005-2010.
Figure 4-6. Least square mean (±1 SE) soil CO$_2$ flux (µmol m$^{-2}$ s$^{-1}$) for Control, Grazed, Pulse, and Press treatments within A) topographic position across a dune from 2005-2010 B) growing season from 2005-2010 and C) growing season from 2011-2014. The Press treatment is not included in panel C as it was discontinued after 2010. Within each panel, bars not connected by the same letter are significantly different (p<0.05).
Figure 4-7. Soil CO$_2$ flux for early and late growing seasons for the number of years that vegetation in the plots were dead. Bars are least square means (±1 SE) from the mixed-model. Bars not connected by the same letter are significantly different (p<0.05). Inset graph is to the mean growing season soil CO$_2$ flux across the number of years dead.
Figure 4-8. Comparison of observed and model predicted mean (±1 SE) seasonal CO$_2$ flux from 2005-2014, combining Control and Grazed treatment data. Figure inset is a regression (red line with 95% CI) of observed and model predicted mean (±1 SE) CO$_2$ flux for each date from 2005-2014.
Figure 4-9. Mean (±1 SE) soil CO₂ flux for A) early and late growing seasons B) topographic position along a dune profile and C) years in the study. Data are from Control and Grazed treatments from 2005-2014. Bars not connected by the same letter are statistically different (p<0.05). Panel D relates using linear regression (±95% CI) the growing season precipitation (April-October) with the model output mean (±1 SE) growing season soil CO₂ flux from 2005-2014 (same as in panel C).
CHAPTER 5 - TOTAL SOIL CARBON AND NITROGEN RESPONSES TO EXPERIMENTAL VEGETATION DISTURBANCES OVER A DECADE IN THE NEBRASKA SANDHILLS

Abstract

The Nebraska Sandhills have undergone several dune mobilization and stabilization cycles in the last 15,000 years. These events are generally linked with changes in climate, most notably increased drought conditions. The mixed-grass prairie vegetation currently stabilizes the sandy soils (~94% sand) by reducing wind erosion and anchoring the soil through root-soil complexes and providing inputs for soil organic carbon (SOC). Because SOC reflects the long-term dynamics and organic matter inputs, measuring total soil carbon (C) and nitrogen (N) will give an indication of the lasting effects of disturbances and changes in plant community composition. The objective of this research was to describe the pre-treatment baseline patterns of total soil carbon and nitrogen in the Sandhills landscape and compare the changes in soil C and N between treatments (Control, Grazed, Pulse, Press) in the Grassland Destabilization Experiment (GDEX) from 2004-2013. I measured total soil C and N in the top 10cm to 30cm of the soil at three points during the ten year experiment (2004, 2008, 2013) across the four treatments and over several topographic positions (ridge, slope, swale). I hypothesized that permanent reduction of vegetation (Press) would severely reduce soil C and N, and periodic disturbances of vegetation (Pulse) would reduce soil C and N compared to Control plots. Pre-treatment data indicated that 40% of the total soil C and N in a 1m soil
profile is located in the top 10cm of soil (948 ± 34 g of C m$^{-2}$; 88 ± 3 g of N m$^{-2}$) and ranges from 0.184-1.792 %C and 0.022-0.163 %N. I determined soil C to be positively associated with grass biomass, and negatively associated with elevation and slope. I found that Press treatments reduced soil C and N by 80% and 82%, mostly through physical wind erosion of the soil, which did not begin until 2009. Contrary to my hypothesis, the Pulse treatment did not show any significant reductions in soil C and N.

The Sandhills grasslands are able to withstand short-term disturbances and retain ecosystem functioning, but it remains to be determined how these responses are altered on longer time scales. The inherent stability and resilience of the Nebraska Sandhills to withstand periodic disturbances and maintain ecosystem properties is critical, especially in the context of predicted climate change impacts (increased temperatures and rainfall variability) for the region.

**Introduction**

Geologic records of sand dune activity in the Nebraska Sandhills are linked to changes in climate, most notably increased occurrences of prolonged and severe drought (Nicholson and Swinehart 2005, Schmieder et al. 2012). During the last 15,000 years, the Sandhills have lost their stabilizing grass cover several times and shifted into a state of dune activity (Loope and Swinehart 2000, Miao et al. 2007b). The most recent occurrence of dune activity occurred 700-1000 years ago during the Medieval Climatic Anomaly, when winds that bring moisture from the Gulf of Mexico shifted, and severe drought became frequent (Sridhar et al. 2006b, Schmeisser et al. 2009, 2010, Schmieder et al. 2011, 2012, Schmeisser McKean et al. 2014). These disturbances reduced prairie
vegetation cover and inputs of carbon belowground, both of which act to stabilize the soil from wind erosion that is prevalent during late winter and early spring.

Recent droughts in the 1930’s and 1950’s failed to reduce the vegetation cover enough to shift the dunes into a mobile state (Weaver 1944, Albertson and Weaver 1944). The evidence from the geologic record and the effects of recent droughts lead to the question of how much disturbance is required to shift the dunes into a mobile state (Schmeisser et al. 2009)? How quickly does the process happen? Understanding the role of vegetation and the carbon balance will provide some answers to the process of how the Sandhills move from a grass-covered, stabilized state to a bare-sand, mobile state.

Sand dune stabilization involves both aboveground primary production which buffers wind erosion, and the belowground organic matter and the grassland root-soil system which provides structure to soils (Schmidt et al. 2011). The warm-season, C₄ dominated grasslands in the Sandhills produce an average of 100-300 g m⁻² yr⁻¹ in aboveground biomass, but on average, 60-70% of annual primary production in grasslands is allocated to belowground biomass every year (Wedin and Tilman 1990). Because of this, more than 95% of C in grasslands is found belowground as soil organic matter (Wedin 2004). Temperate grasslands are an important sink for soil organic carbon (SOC) and are estimated to contain 105-295 Gt of C in the top meter of soil (Stockmann et al. 2013). The amount of SOC in a particular site reflects the long-term dynamics of inputs to the soil, which are usually correlated with net primary production, and outputs through decomposition and leaching (Zhou et al. 2009). SOC generally declines with increased temperature and precipitation (Epstein et al. 2002, Craine and Gelderman 2011,
Murphey 2014), declines with increased disturbance (Munson et al. 2012), and can fluctuate based on changes in plant community composition and traits (De Deyn et al. 2008). Because SOC reflects the long-term vegetation dynamics and organic matter inputs, measuring total soil C and N will give an indication of the lasting effects of disturbances and changes in plant community composition.

In the Sandhills, the coarse textured sandy soils (>94% sand) do a poor job of organic matter protection and generally have low C content in soils at sand dune ridges (0.33-0.51 %C; Wang et al. 2008). The organic C and N in the soil is mainly located in the top 10 cm and declines rapidly with depth (Hellerich 2006). Vegetation cover is hypothesized to be the primary factor that reduces wind erosion (Li et al. 2007), but the thin layer of organic matter and plant roots also play an important soil in water and wind erosion (Lal 2005). Loss of the vegetation cover and decreased SOC can lead to blowouts (generally 0.1-0.3 hectares; Stubbendieck et al. 1989), but the transition to widespread dune mobilization in unclear. Decreases in total soil C and N can occur through two pathways: decreased functionality of the grassland ecosystem, which leads to a net negative C balance, and physical erosion of soil. Decreased functionality of the grassland ecosystem leads to reduced C and N inputs into the soil, while shifts in the microclimate can alter soil respiration, soil moisture and temperature, and soil nitrates (Turner et al. 1997, Vargas et al. 2012, Imer et al. 2013, Li and Guo 2014). Physical erosion reduces total soil C and N by removing the top soil layers and exposing lower soil profiles, which have a lower total soil C and N content.
The objective of this research was to: 1) Describe the variation of and factors affecting total soil C and N across the landscape before treatment application and 2) Compare the changes in total soil carbon and nitrogen between treatments in the Grassland Destabilization Experiment (GDEX) from 2004-2013. I describe the spatial variation in total soil C and N, assess the response of the Sandhills grasslands to the different vegetation disturbances, and site differences based on topography. I hypothesized that the percent total soil C and N (%C and %N) will be negatively correlated with topographic position (~sand content) and positively correlated with the proportion of vegetation composed of grasses. I also hypothesized that total soil C and N will decrease over time as inputs of organic matter are reduced. As aboveground and belowground plant biomass is reduced, soil respiration will continue, producing a net negative C balance. Treatments that reduced plant biomass permanently and experienced high erosion (Press treatment) should exhibit the greatest reduction in total soil %C and %N, and treatments that reduced biomass temporarily with little to no erosion (Pulse treatments) should produce reduced total soil %C and %N compared to Control treatments, but not reach the extent of Press treatments.

Methods

Study Site & Experimental Design

This research was conducted within the Grassland Destabilization Experiment (GDEX), a landscape-scale experiment examining the role of vegetation in dune stabilization, started in 2004 at UNL’s Barta Brothers Ranch (BBR) in the eastern
Sandhills (Wang et al. 2008; Sridhar and Wedin 2009). Mean annual temperature at BBR is 8.1 °C and mean annual precipitation is 576 mm (Wang et al. 2009a), where 80% of precipitation falls during the growing season between April and September (Schacht et al. 2000). In general, around 90% of the surrounding landscape is composed of upland dunes and dry interdunal areas (swales) dominated by warm-season prairie grasses, while the remaining 10% is subirrigated wet meadows and wetlands. The GDEX site itself is categorized as upland prairie, with no subirrigated wet meadows. The dunes are composed of Holocene eolian sand deposits which overlie Quaternary and/or Pliocene alluvial sand and silt (Wang et al. 2009a). Across the Nebraska Sandhills, the dunes are generally oriented from the northwest to the southeast, and dune heights can be as large as 40m high, 825m long, and 1220m wide (Bleed and Flowerday 1998). At BBR, dunes are generally smaller, ranging from 5-20m in height (Wang et al. 2009a) and slopes are 5-15%. Topographic position and aspect across a dune profile affect community composition, with interdunal areas (15% of landscape) having a higher proportion of switchgrass (*Panicum virgatum*), bluegrass (*Poa pratensis*), blue grama (*Bouteloua gracilis*), and sedges (*Carex eleocharis*). The dune slopes and ridges (85% of landscape) are composed of warm-season, C₄ grasses such as prairie sandreed (*Calamovilfa longifolia*) and sand bluestem (*Andropogon hallii*), which are generally located on south facing slopes, while the north slopes contain more cool-season, C₃ grasses such as needle and thread grass (*Hesperostipa comata*), prairie june grass (*Koeleria macrantha*), and the shrub leadplant (*Amorpha canescens*) (Schacht et al. 2000, Milby 2011). Interdunal swale locations also have greater soil carbon content (0.51%) compared to dune slopes and
ridges (0.33%) in the top 30cm, with slightly less sand in the top 10cm of swale locations (91.2% sand) compared to dune slopes and ridges (94.4% sand). Interdunal swale A horizons extended to an average depth of 22.8cm (range 6-41cm), while A horizons on the dune ridges extended to an average depth of 11.8cm (range: 6-21cm; Wang et al. 2008).

The GDEX project was set up in 2004 with ten circular plots, 120m in diameter (1.1 hectares total area), which span the profile of one sand dune, from swale to dune top to swale. Within each plot, there were 22 subplots, with varying locations across elevation, aspect, and slope. Each of the ten plots was assigned to one of five management treatments, which range from continuous grass cover to bare sand. Treatments included two Control plots (with no grazing), two Grazed plots (deferred rotational grazing), two Press plots (no grazing, plant growth prevented with herbicide), two Pulse plots (no grazing), and two Aggressive plots (no grazing, vegetation killed with herbicide and disked; treatment not used in this analysis). Press and Pulse treatments were chosen to examine the response of the Sandhills grassland ecosystem to experimental vegetation disturbances and provide insights into the stability and resilience of the sand dune grasslands to permanent and periodic vegetation suppression (Bengtsson et al. 2003, Collins et al. 2011). Plots in the Pulse treatment are on a 3-year schedule, with vegetation killed with herbicide glyphosate in the first year (reapplications to prevent regrowth), followed by two years of recovery. Vegetation in the Pulse plots was killed (without physical disturbance) in 2005, 2008, 2011, and 2014, but allowed to recover during intervening years (see Chapter 1 for detailed methods). The Press
treatment is similar to the Pulse treatment, but does not allow for vegetation recovery. Vegetation in the Press treatment plots was killed with herbicide glyphosate in 2005, with further reapplications as necessary to prevent vegetation regrowth (see Chapter 1 for detailed methods). Vegetation was kept dead from 2005-2010, at which point the Press treatment was discontinued because the plots were fully mobilized. The Press plots were subsequently planted with native shrubs and grasses beginning in 2011 to facilitate dune stabilization. Plots in the Grazed treatment were part of the deferred rotational grazing study at Barta Brother Ranch (Schacht et al. 2010, 2011). From 2004-2014, the plots in the DRG study pastures were grazed with 60-132 cow-calf pairs (1.5 AUE) over either three periods (2-14 days) or one period (30-42 days) during the grazing season (15 May – 15 October). Stocking rates were moderate, varying from 1.65-2.12 AUM ha\(^{-1}\) (see Chapter 1 for more details).

**Sampling Procedure**

Soil cores were pulled from every subplot (20-22 total in each plot) on June 15\(^{th}\) 2004, October 20\(^{th}\) 2008, and July 8\(^{th}\) 2013. Soils were cored in every plot except the plots in the Aggressive treatment, which had become mobile, blowing sand dunes by 2006. Within each plot (8 total used), a series of subplot pins (20-22) were distributed across the dune profile to incorporate differences in dune elevation, slope, and aspect. At each subplot location, soils were cored from four locations around the subplot pin, at a distance of 1 m from the pin. Soils were cored using a ¾” diameter corer to a depth of 30 cm and then divided into depths from 0-10cm, 10-20cm, and 20-30cm. Each of the four sub-samples at each subplot pin was divided this way, and then each depth was
aggregated together, thus there were three samples (depths) per subplot. In 2004 soils were cored to a depth of 100 cm (additional depths of 30-50cm, 50-75cm, and 75-100cm in select plots), in 2008 only the top 10cm were cored, and in 2013 0-30cm were cored. Despite the differences in depth, the top 10 cm of the soil was cored in each year (2004, 2008, and 2013). Soil samples were air dried and passed through a 1mm sieve to remove root and leaf fragments. Samples were ground to homogenize and then oven dried at 60 °C. The ground, dried samples were then sent to the Ecosystems Analysis Laboratory at the University of Nebraska – Lincoln and were analyzed for total soil Carbon and Nitrogen (% C and % N) using an Costech ECS 4010 auto-analyzer.

In 2004, soil texture analysis (percent sand, silt, and clay) of the top 10cm was conducted at selected subplot pins (Wang et al. 2009b), along with clipping aboveground biomass at every subplot during the soil sampling on June 15th, 2004. Aboveground biomass was hand-clipped using a 0.25m² frame. Biomass samples were sorted into functional groups (grass/forb/shrub/cacti) and dead fractions. Aboveground biomass samples were oven dried for 48 hours at 60 °C and then weighed (see Chapter 2 for detailed methods).

**Statistical Analysis**

Baseline levels and patterns in total soil C (%C) was analyzed using all 0-10cm data from 2004, as treatments were not implemented until the spring of 2005. A multiple linear regression model (JMP 11.0.0) was used to analyze the response of %C by incorporating the continuous variables of grass, forb, shrub, cacti, and dead aboveground biomass, along with soil texture (% sand), percent elevation (subplot elevation divided by
the elevation of the highest subplot within a plot), slope, and aspect class (N,E,S,W).

Model selection was conducted using stepwise methods with p-value thresholds (probability to enter/leave model = 0.25) for model comparison.

Changes in percent total soil C, N, and C:N (%C / %N) ratio were analyzed using a mixed effects model (JMP 11.0.0) with treatment, topography, depth, and year treated as fixed effects. Topographic position was treated as a categorical variable where subplot pins in each plot were allocated to ridge, slope, or swale topographic positions within in the plot. Although the distribution of subplots into topographic positions was unbalanced within and between plots, across all plots 30.1% of subplots were located in ridge positions, 37.5% in slope positions, and 32.4% in swale positions. Random effects included the specific plot and subplot nested inside topography and treatments. Multiple comparisons between treatment, topography, depth, and year were done using Tukey’s HSD. A mixed effects model was run using only the data from the top 10 cm of soil that was cored in each year (2004, 2008, and 2013). A second mixed effects model was run comparing responses of soil carbon, nitrogen, and C:N for the 10-30cm depths that were cored in 2004 and 2013. Model assumptions were checked by comparing residuals to predicted values, Q-Q plots, and scale locations plots.

Results

Variability of Total Soil C and Associated Factors

In general, total soil C (%) and N (%) declined as soil depth increased (Figure 5-1). Across all topographic positions, percent total soil C varied from 0.18-1.79 %C in
the top 10cm, 0.14-0.64 %C from 10-20cm, and 0.09-0.53 %C at 20-30cm. Percent total soil N was similar, ranging from 0.02-0.16 %N in the top 10cm, 0.02-0.05 %N from 10-20cm, and 0.01-0.45 %N from 20-30cm. At the beginning of the experiment in 2004, across all plots, swale locations contained 38% and 35% more total soil C and N in the top 10cm (0.81 %C; 0.07 %N) compared to slope locations (0.59 %C; 0.05%N), and 75% and 67% more total soil C and N compared to ridge locations (0.46 %C; 0.04 %N; Figure 5-1). Averaged across all topographic positions, 40% and 36% of the total soil C and N is located in the top 10 cm. A majority of the total soil C (75.4%) and N (70.9%) is accounted for in the top 30cm in a 1m soil profile (Figure 5-1).

Differences in total soil C for the 0-10cm soil depth were affected by the slope and elevation of the subplot location and the associated vegetation. Model selection resulted in a multiple linear regression with the variables aboveground grass biomass, aboveground forb biomass, aboveground shrub biomass, slope, and percent elevation (Table 5-1). The aboveground grass biomass, slope, and percent elevation were significant in their ability to explain the variation in percent total soil C. Total soil C was positively associated with aboveground grass biomass, while negatively associated with elevation and slope (Figure 5-2). The stepwise model selection removed percent sand as a model variable most likely because there were relatively few data points (soil texture was not analyzed for every subplot), but there was a negative relationship between total soil C and percent sand (Figure 5-2). Percent total soil C was significantly higher in swale locations (mean=0.81 %C) compared to slope (p<0.0001; 0.60 %C) and ridge (p=0.0036; 0.44 %C) locations, along with significant differences (p=0.0229) between
slope and ridge locations (Figure 5-1). Percent total soil C was also significantly lower in subplot locations with a steeper slope (>10%; mean=0.47 %C) compared to subplot locations with flat (0-5%; p=0.0007; 0.70 %C) or moderate (5-10%; p=0.0049; 0.64 %C) slopes (Figure 5-2).

Changes in Total Soil C, N, and C:N from 2004-2013

The mixed effects model analyzing the top 10cm of soil in 2004, 2008, and 2013 for %C, %N, and the C:N ratio did not have any three way interactions between topography, treatment, and year (Table 5-2). The mixed model analysis was able to account for 50-70% of the variation in the %C, %N, and the C:N ratio (Table 5-2). The second mixed effects model analyzing the soil from the 10-30cm depths between 2004 and 2013 showed significant three way interactions between treatment × topography × year (Table 5-3), and between treatment × depth × year (Table 5-3). The mixed model analysis accounted for 67-81% of the variation in the data (Table 5-3).

0-10cm Depth (2004, 2008, and 2013)

There were significant differences in total soil C and N in the top 10 cm of soil over the course of the experiment. At the beginning of the experiment in 2004, the mean percent total soil Carbon varied from 0.53-0.72 %C in all plots before treatments were implemented (Figure 5-3). By 2008, mean total soil C in the top 10cm had not changed drastically, with the Press treatment reduced to a mean of 0.47 ± SE 0.05 %C, marginally different from the 2004 Press treatment (p=0.0892) and the 2004 Pulse treatment (p=0.0781). By 2013, all treatments experienced slightly reduced total soil C, but the
only significant difference occurred in the Press treatment, with a mean of 0.12 ± 0.05 %C, significantly different from all other treatment-year combinations (Figure 5-3).

The responses of total soil N in the top 10cm were more variable, but similar in their general pattern compared to total soil C (Figure 5-3). At the beginning of the experiment, mean percent total soil N ranged from 0.05-0.07 %N in the top 10cm of soil. By 2008, in the 4th year of the experiment, the Press treatment was reduced to a mean of 0.04 ±0.01 %N, significantly different from the 2004 measurement. In 2013, total soil N in Press treatment was reduced to a mean of 0.01 ±0.01 %N, significantly different from all other treatment-year combinations (Figure 5-3). Overall, the Pulse treatment had the highest mean total soil N in the top 10cm of soil for each year over the course of the experiment.

The soil C:N ratio in the top 10cm of soil showed the only significant differences in a topographic effect over time (Table 5-2). The soil C:N ratio started out on average between 10.44-10.91, with swale locations having higher soil C:N ratios than slope locations, and slope locations higher than ridge locations (Figure 5-4). This trend was the same in 2008, but in 2013, the pattern changed. All topographic positions showed increased soil C:N ratios by 9-17%, although not all increases were significantly different. The starkest difference was in the ridge locations, where the soil C:N ratio increased to a mean of 12.66 ± 0.36, which was significantly different than the soil C:N of ridge locations in 2004 and 2008 (Figure 5-4). Slope locations in 2013 were also significantly different from ridge locations in 2004 and 2008.
ANOVA results indicate significant differences were present in the top 10cm of soil between treatments at varying topographic positions (Table 5-2). The general pattern is that total soil C decreases as topographic position increases (Figure 5-5). The Press treatment had the lowest total soil C across all topographic positions, although not significantly different. Total soil C responses for Control and Pulse treatments in swale locations were significantly higher than almost all other treatment × topography combinations (Figure 5-5). The Control × swale interaction was marginally significant compared to the Grazed × swale (p=0.0973) and the Grazed × slope (p=0.0694) interaction. The Pulse × swale interaction was also marginally different compared to the Grazed × swale combination (p=0.0580).

There were significant differences in total soil N in the top 10cm of soil (Table 5-2). As with the treatment effects over time, the responses of total soil N for the treatment-topography interaction is similar to the total soil C pattern. As before, the general trend is decreasing total soil N as topographic position increases (Figure 5-5). Total soil N was more variable in swale locations compared to slope and ridge locations. Again, Control (0.08 ± 0.01 %N) and Pulse (0.08 ± 0.01 %N) treatments in swale locations had significantly higher total soil N compared to almost all other treatment × topography combinations (Figure 5-5). For the combinations in which they were not significantly different from at the p≤0.05 level, they were marginally significantly different from at the p≤0.10 level.
**10-30cm Depth (2004 vs 2013)**

A second mixed model analysis of 10-30cm soil data showed significant differences for treatment by topographic interactions over time in total soil C and N responses (Table 5-3). Similar to the 10cm soil data, total soil C and N decreased as elevation (topography) increased (Figure 5-6). In 2004, mean total soil C (10-30cm) ranged from 0.25-0.35 %C in swale locations and 0.21-0.24 %C in ridge locations. By 2013, total soil C had significantly decreased (15-20% loss) relative to 2004 values for Control, Grazed, and Pulse treatments in all topographic locations (Figure 5-6). In the Press treatment, total soil C decreased by 50-70% in all topographic locations, significantly different from all other treatment × topography combinations in 2013 (Figure 5-6).

There was a similar trend in the response of total soil N in the 10-30cm soil depth (Table 5-3). In 2004, mean percent total soil N ranged from 0.02-0.04 %N and was more variable in swale locations compared to slope (0.03 %N) and ridge locations (0.02-0.03 %N; Figure 5-6). Again, by 2013 the total soil N for Control, Grazed, and Pulse treatments significantly decreased (26-38% reduction) in all topographic locations compared to 2004 values. The Press treatment decreased by 56%, 67%, and 76% respectively in swale, slope, and ridge locations (Figure 5-6), significantly different from all other treatment × topography combinations in 2013.

The second mixed model analysis also indicated significant differences in total soil C and N for the treatment × depth (10-20cm, 20-30cm) interactions from 2004 to 2013 (Table 5-3). As before (Figure 5-1), total soil C and N decreased as depth
increased. Mean percent total soil C ranged from 0.28-0.32 %C in the 10-20cm depth across all treatments in 2004 and 0.21-0.24 %C in the 20-30cm soil profile (Figure 5-7). By 2013, total soil C from both 10-20cm and 20-30cm depths in Control, Grazed, and Pulse treatments slightly decreased, but it was not significantly different compared to the 2004 levels. However, the Press treatment lost an average of 61% of the total soil C from the 10-20cm depth profile, and 56% of the total soil C from the 20-30cm soil profile (Figure 5-7).

The response of total soil N for treatment × depth from 2004 to 2013 was very similar to total soil C (Table 5-3, Figure 5-7). Mean percent total soil N in the 10-20cm depth ranged from 0.028-0.033 %N across all treatments in 2004, and 0.022-0.026 %N in the 20-30cm depth. Again, there was a sharp decrease in total soil N for the Press treatment in both the 10-20cm soil profile (68% decline) and the 20-30cm soil profile (65% decline; Figure 5-7). All other treatments in 2013 (Control, Grazed, Pulse) were significantly reduced in total soil N from the previous measurements in 2004 for both the 10-20cm and the 20-30cm soil profiles (Figure 5-7).

**Discussion**

The goal of this research was to describe the spatial variation in soil carbon and compare the changes in soil carbon and nitrogen between treatments from 2004-2013 in the Grassland Destabilization Experiment (GDEX). Measurements of total soil C and N at three points during the ten year experiment were analyzed to determine the effects of treatments, topography, depth, and time. Total soil C was positively associated with aboveground grass biomass, and negatively associated with slope and elevation. In
general, the data support my hypotheses that (1) in the predominately sandy soils (~94% sand) total soil C and N decreased as topography increased and (2) permanent reduction in plant biomass produced a significant reduction in total soil C and N. However, my hypothesis that the total soil C and N in the Pulse treatment would be reduced over several pulse cycles compared to Control and Grazed treatments was not supported by the data (Figure 5-3, Figure 5-5, Figure 5-6, and Figure 5-7).

Overall, the mean percent total soil C over ten years in the top 30cm of ridge (0.25 %C) and swale (0.51 %C) locations in Control plots was similar to values reported for other experiments at the GDEX site for ridge (0.33 %C) and swale (0.51 %C) locations (Wang et al. 2008). My results are also similar to other Sandhills site, such as the Nebraska National Forest at Halsey, where prairie soils contained 0.52 %C in the top 30cm (Hellerich 2006). Soils at subirrigated meadows at Barta Brothers Ranch (Mousel et al. 2007) contain 1.7 %C and 0.15 %N in the A horizons (0-10cm) and 4 %C and 0.4 %N in C horizons (10-30cm). These values are higher than both %C and %N of A horizon soils in swale locations for Control (0.95 %C; 0.08 %N) and Grazed (0.61 %C; 0.05 %N) treatments as well as C horizon swale soils (Control: 0.58%C; 0.05%N) (Grazed: 0.57%C; 0.05%N). The %C and %N in the swale locations at the GDEX site are likely lower as subirrigated meadows at Barta contain a higher proportion of C₃ species (Mousel et al. 2007) and the higher and more seasonally consistent water availability produces greater biomass, thus increasing %C and %N in soils. Variation in total soil carbon before the start of the experiment in 2004 was driven by the amount of grass biomass and elevation. This is similar to other range sites at BBR (Schacht et al.
2000) where soil organic matter was not different between dune tops and north and south facing slopes at BRR (0.40-0.46 %C), but was significantly greater in interdunal areas (0.87 %C). My results support these findings that although aspect may affect the vegetation composition over a sand dune, it has little effect on the soil carbon.

**Vegetation Effects on Total Soil C and N**

Total soil C in the Press treatments did not show any significant reductions until soils were sampled in 2013, although there was a significant loss in %N by 2008 as there was no plant growth to retain nitrogen. By 2013, the dunes in the Press treatment had been fully mobilized since 2009, and an average of 1.22m of soil had been eroded from the dune tops (Figure 5-8). The total soil C in the Press treatment in 2013 was similar in all three depths, with an average of 0.11 %C within the 0-30cm soil depth (Figure 5-3, Figure 5-7). Total soil N was similar across all depths in the Press treatment, averaging 0.01 %N (Figure 5-3, Figure 5-7). Total soil N in the Press treatment was significantly reduced by 2008 in the top 10cm (Figure 5-3), indicating that total soil N was being mineralized and then lost from the system as there was no plant uptake of N due to the treatment effects. There were no significant differences in total soil C between treatments in 2008, but the Press treatment did experience a 21% drop in mean total soil C from 2004 (Figure 5-3) with an average erosion of 5.71cm, 3.49cm, and 0.84cm of soil in ridge, slope, and swale locations respectively (Figure 5-8). This indicates that some of the soil C was lost as there was a net negative C balance through reduced inputs between 2004 and 2008 (27% of the total C lost). However, a majority (73%) of the soil C that was lost from 2004-2013 was lost primarily through wind erosion of the top soil layers,
starting in the spring of 2009 (Figure 5-8). Wind erosion in the Sandhills blows sand from
the northwest to the southeast in the winter, and from the southeast to the northeast in the
summer (Schmeisser et al. 2010). Because of the change in seasonal wind direction,
mobile sand dunes are consistently turned over and is the reason for the homogenous total
soil C and N values in each soil depth for the Press treatment in 2013 (Figure 5-3, Figure
5-7). These results are similar to other highly degraded sandy grasslands, where erosion
severely reduced total soil C (111-474%) and N (60-198%) (Li et al. 2009).

I hypothesized that the total soil C and N in the Pulse treatment would be reduced
compared to the Control and Grazed treatments, and that the treatment effects would not
be as severe as the Press treatment. The data did not support this hypothesis and
indicated that there were no significant differences in total soil C or N between the Pulse
treatment and the Control and Grazed treatments. This result held for comparisons of
treatments over time (Figure 5-3), within topographic positions (Figure 5-6), and within
soil profile depth (Figure 5-7). Not only were Pulse treatments not experiencing any soil
erosion (±0.5cm regardless of topographic position by 2013) compared to Control
treatments (±1cm), but the total soil C and N responses suggest that there has not been a
great loss in belowground ecosystem functioning (Figure 5-2, Figure 5-5, Figure 5-6, and
Figure 5-7). Consequently, there was little net change in the height of the soil surface of
the Pulse treatment (mean = 0.42 ± 1.13cm) compared to the Control treatment (mean =
1.03 ± 0.49cm) by 2013.

An intriguing response measured across all treatments, topographic positions, and
depth was a trend in decreased total soil C and N by 2013, compared to levels in 2008
and 2004 (Figure 5-3, Figure 5-6, Figure 5-7). The magnitude of reductions in both total soil C and N over time are reflected in the altered C:N ratio of the soil. In the top 10cm of the soil, C:N ratios increased for all topographic locations in 2013 (Figure 5-3). Total soil C in the top 10 cm was reduced by an average of 27%, but total soil N in the top 10 cm was reduced by an average of 36%, thus increasing the soil C:N ratio. Variation in sampling and lab analysis were ruled out, so this response could be driven by exceptional drought the previous year in 2012 where daily mean temperatures increased by +1.9 – 6.1 °C during the summer months, and annual precipitation was 47% (257mm) of the ten year average (Mallya et al. 2013, Hoerling et al. 2014). The persistent and severe drought reduced plant growth and uptake of mineralized N, reducing the amount of N in roots. A possible outcome is that reduced uptake and storage led to soil N building up, and then excess mineralization led to N leaching from the ecosystem in the following year’s spring rains (Schwinning et al. 2004), further reducing the N in the soil and increasing the soil C:N ratio.

**Estimated Total Soil C and N Stocks in the Sandhills**

I have presented total soil C and N values as %C and %N and not on a g m^{-2} basis because bulk density was not measured concurrently with soil core sampling. Using an average bulk density of 1.53 g cm^{-3} for the top 30cm of Sandhills soils (Hellerich 2006), the I estimated the mean (±1SE) soil C and N stock in the top 30cm for the Control treatment over ten years to be 1631 ± 67 g of C m^{-2} and 148 ± 6 g of N m^{-2}. The Grazed treatment was similar with a mean of 1598 ± 44 g of C m^{-2} and 141 ± 4 g of N m^{-2}. Across the globe, grassland ecosystems range from 2,000-8,000 g of C m^{-2} and 100-600 g
of N m\(^{-2}\) in the top 30cm of soil (Parton et al. 1993) depending on the vegetation, soil
texture, temperature, and precipitation. The shortgrass steppe grasslands to the west of
the Sandhills contain on average 1,500-2,500 g of C m\(^{-2}\) and 100-200 g of N m\(^{-2}\) (Parton
et al. 1987). In more southern regions of the Great Plains, soil Carbon and Nitrogen
range from 1,500-2,600 g of C m\(^{-2}\) and 130-220 g of N m\(^{-2}\) in the top 15cm of sandy
loam soils (Zhou et al. 2009), while the tallgrass prairies to the south and east of the
Sandhills, with silty clay loam soils contain 4,500-6,500 g of C m\(^{-2}\) and 300-600 g of N
efforts have estimated the total soil C and N in the Sandhills region at 2,000-3,500 g m\(^{-2}\)
and 100-400 g m\(^{-2}\) of soil Nitrogen at 0-20cm depth (Parton et al. 1987, Burke et al.
1997). My soil C and N values are lower than predicted, and are more similar to the total
soil C and N content in the shortgrass prairie in the western Great Plains than the tallgrass
prairie to the east. The small pool of total soil C and N, compared to other grasslands, is
likely constrained by not only precipitation and temperature, but more importantly the
sandy soil texture (Burke et al. 1989, Hassink 1992, Epstein et al. 2002). The soils at this
Sandhills site contain 90-98% sand (Figure 5-2), which limits the ability for soil organic
carbon to build up as the sandy soils allow for greater decomposition rates. This also
limits the potential for the semi-arid Sandhills to be a net sink for C sequestration
(Munson et al. 2012).
Conclusion

Permanently suppressing the aboveground vegetation (Press treatment) in a Nebraska Sandhills grassland produced significant reductions in total soil C and N after ten years, mainly through physical removal of the top soil layers from wind erosion. The responses of total soil C and N show that aboveground vegetation cover and the root-soil association plays a bigger role in sand dune stability than SOC. I expected that periodic disturbance of aboveground vegetation (Pulse treatment) would lead to reduced total soil C and N, as the Pulse treatment would reduce inputs of C and N to the soil every third year. The lack of reduction in total soil C and N in the Pulse treatment indicates the ability of the grassland ecosystem to recover from periodic disturbances (Albertson and Weaver 1944, Hoover et al. 2014a) and demonstrates the inherent stability of the Sandhills (Stubbendieck and Tunnell 2008) to short term disturbances. However, this experiment was conducted over an ecologically short-term time scale (~10 years), so the inherent stability and resilience of the Sandhills to short-term disturbances may be different over longer time frames. On millennial time scales, large scale, long-term severe drought is the main driver of the stability of the Sandhills, and these disturbances have overcome the resilience of the Sandhills several times in the last 10,000 years (Miao et al. 2007b). The ability of the Sandhills grassland ecosystem to withstand periodic disturbance will become more important as predicted climate change impacts of increased temperatures and rainfall variability (Romero-Lankao et al. 2014) emerge in the next century.
Figures & Tables

Table 5-1. Combined effects tests and parameter estimates of a multiple linear regression model (stepwise) for percent total soil Carbon across all subplots in 2004 (pre-treatment). Numbers in bold indicate significant (p≤0.05) model effects. Elevation (%) is the ratio of the elevation of a subplot to the elevation of the highest subplot within a plot (a single dune profile).

| Source  | Sum of Squares | F Ratio | Prob > F | Estimate | Std Error | t Ratio | Prob >|t| |
|---------|----------------|---------|----------|----------|-----------|---------|-------|-------|
| Intercept | —              | —       | —        | 1.0495   | 0.1040    | 10.09   | <0.0001 |
| Grass   | 0.272          | 5.38    | 0.0217   | 0.0013   | 0.0006    | 2.32    | 0.0217 |
| Forb    | 0.002          | 0.05    | 0.8258   | 0.00019  | 0.0008    | 0.22    | 0.8258 |
| Shrub   | 0.025          | 0.49    | 0.4851   | 0.00027  | 0.0004    | 0.70    | 0.4851 |
| Slope   | 0.646          | 12.80   | 0.0005   | -0.0143  | 0.0040    | -3.58   | 0.0005 |
| Elevation | 2.103        | 41.69   | <0.0001  | -0.6206  | 0.0961    | -6.46   | <0.0001 |

Observations = 159; Adjusted $R^2$=0.35; Error DF = 153
Table 5-2. Mixed model summary of fit and ANOVA results for percent total soil carbon, nitrogen, and the C:N ratio in the top 10 cm of soil for 2004, 2008, and 2013. Numbers in bold show significant main effects or interactions (p≤0.05).

<table>
<thead>
<tr>
<th>Source</th>
<th>Total Soil C (%)</th>
<th>Total Soil N (%)</th>
<th>Soil C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Year</td>
<td>35.16</td>
<td>&lt;0.001</td>
<td>82.55</td>
</tr>
<tr>
<td>Trt</td>
<td>8.11</td>
<td>0.0104</td>
<td>7.08</td>
</tr>
<tr>
<td>Topo</td>
<td>22.13</td>
<td>&lt;0.0001</td>
<td>19.11</td>
</tr>
<tr>
<td>Year × Trt</td>
<td>13.56</td>
<td>&lt;0.0001</td>
<td>14.80</td>
</tr>
<tr>
<td>Year × Topo</td>
<td>0.44</td>
<td>0.7779</td>
<td>0.76</td>
</tr>
<tr>
<td>Trt × Topo</td>
<td>3.30</td>
<td>0.0260</td>
<td>2.91</td>
</tr>
<tr>
<td>Year × Trt × Topo</td>
<td>0.79</td>
<td>0.6585</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Table 5-3. Mixed model summary of fit and ANOVA results for percent total soil carbon, nitrogen, and the C:N ratio of soil from 10-30cm in 2004 and 2013. Numbers in bold show significant main effects or interactions (p≤0.05). Italicized numbers indicate marginal significance (p<0.10).

<table>
<thead>
<tr>
<th>Source</th>
<th>Adjusted R²</th>
<th>Total Soil C (%)</th>
<th>Total Soil N (%)</th>
<th>Soil C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Year</td>
<td>0.75</td>
<td>74.86</td>
<td>0.0009</td>
<td>263.59</td>
</tr>
<tr>
<td>Trt</td>
<td>7.28</td>
<td>0.0270</td>
<td>11.24</td>
<td>0.0071</td>
</tr>
<tr>
<td>Depth</td>
<td>255.27</td>
<td>&lt;0.0001</td>
<td>260.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Topo</td>
<td>8.99</td>
<td>0.0050</td>
<td>7.08</td>
<td>0.0106</td>
</tr>
<tr>
<td>Year × Topo</td>
<td>0.93</td>
<td>0.3961</td>
<td>1.08</td>
<td>0.3391</td>
</tr>
<tr>
<td>Trt × Topo</td>
<td>0.53</td>
<td>0.7761</td>
<td>0.56</td>
<td>0.7549</td>
</tr>
<tr>
<td>Topo × Depth</td>
<td>1.06</td>
<td>0.3480</td>
<td>1.34</td>
<td>0.2634</td>
</tr>
<tr>
<td>Year × Trt</td>
<td>10.90</td>
<td>0.0208</td>
<td>8.61</td>
<td>0.0305</td>
</tr>
<tr>
<td>Year × Depth</td>
<td>0.15</td>
<td>0.7013</td>
<td>1.03</td>
<td>0.3104</td>
</tr>
<tr>
<td>Trt × Depth</td>
<td>3.77</td>
<td>0.0108</td>
<td>3.97</td>
<td>0.0082</td>
</tr>
<tr>
<td>Year × Trt × Depth</td>
<td>4.84</td>
<td>0.0025</td>
<td>3.91</td>
<td>0.0089</td>
</tr>
<tr>
<td>Year × Trt × Topo</td>
<td>2.75</td>
<td>0.0124</td>
<td>2.83</td>
<td>0.0102</td>
</tr>
<tr>
<td>Year × Topo × Depth</td>
<td>0.47</td>
<td>0.6249</td>
<td>0.56</td>
<td>0.5705</td>
</tr>
<tr>
<td>Year × Trt × Topo × Depth</td>
<td>1.13</td>
<td>0.3447</td>
<td>1.19</td>
<td>0.3094</td>
</tr>
</tbody>
</table>
Figure 5-1. Mean (±1 SE) total soil A) carbon (%) and B) nitrogen (%) for swale, slope, and ridge topographic positions at six depths in the soil profile (0-10cm, 10-20cm, 20-30cm, 30-50cm, 50-75cm, and 75-100cm). Data are averaged from all plots in 2004, as treatments were not applied until 2005.
Figure 5-2. Linear regressions of total soil carbon (%) and A) aboveground grass biomass (g m$^{-2}$) B) percent elevation C) percent slope and D) percent sand. Data are from June 15th, 2004 with total soil carbon and percent sand from 0-10cm soil cores. Percent elevation was determined by dividing the subplot elevation by the highest subplot elevation within the plot (a single dune profile). Panel D (percent sand) has fewer data points as soil texture was not analyzed in every subplot. Lines represent the linear fit with 95% confidence intervals.
Figure 5-3. Mean (±1SE) percent total soil A) carbon and B) nitrogen from 0-10cm for all treatments during three points of the 10-year experiment. Asterisks indicate values that are significantly different (p≤0.05) from all other treatments within a year.
Figure 5-4. Mean soil C/N ratio (±1SE) from 0-10cm of the three topographic positions in three different years over the course of the 10-year experiment. Bars not connected by the same letter are significantly different (p≤0.05).
Figure 5-5. Mean (±1SE) percent total soil A) carbon and B) nitrogen from 0-10cm for the three topographic positions within treatments, with data averaged over all years (2004, 2008, and 2013). Bars not connected by the same letter are significantly different (p≤0.05).
Figure 5-6. Panels A and B are mean (±1SE) percent total soil C for treatment × topography combinations in 2004 (A) and 2013 (B), averaged across 10-30cm depths. Panels C and D are mean (±1SE) percent total soil N for treatment × topography combinations in 2004 (C) and 2013 (D), averaged across 10-30cm depths. Asterisks indicate significant differences (p≤0.05) between treatments within topographic positions.
Figure 5-7. Panels A and C are mean (±1SE) percent total soil carbon for year × treatment combinations for the A) 10-20cm and C) 20-30cm depths. Panels B and D are mean (±1SE) percent total soil nitrogen for year × treatment combinations for the B) 10-20cm and D) 20-30cm depths. Asterisks indicate significant differences (p≤0.05) between treatments within years.
Figure 5-8. Change in soil surface for the Press treatment averaged across swale, slope, and ridge locations. Lines were created by plotting a smoothing spline function to the data points (SigmaPlot v13). Vertical lines indicate the three points of soil sampling in 2004, 2008, and 2013.
CHAPTER 6 - CONCLUSION: BELOWGROUND ECOLOGICAL FUNCTIONG CONTROLS SAND DUNE SURFACE STABILITY

On millenial timescales, the world’s great sand dune landscapes (ergs) are dynamic, switching from vegetated to non-vegetated states (Thomas et al. 2000, Miao et al. 2007b, Yang et al. 2012). Climate is a major driver of these transitions, which are associated with large wind shifts and reduced moisture transport (Sridhar et al. 2006b). However, ecological processes mediate these climatic impacts as sand dune systems transition to alternative states (Ponce Campos et al. 2013, Monger et al. 2015). Sand dune destabilization is sensitive to thresholds of aboveground vegetation (Li et al. 2005, 2007), yet belowground processes are largely ignored (Bardgett and van der Putten 2014). Here I provide results from a ten-year study in a semi-arid sand dune grassland, on the above and belowground ecological responses to experimental vegetation disturbances, which show belowground ecological processes as the basis of resilience. I found that there is a time lag (4-5 years) between disruption of aboveground vegetation and loss of belowground functioning, with sand dune surface destabilization accelerating after belowground functioning was reduced below critical thresholds. Additionally, pulse-recovery vegetation disturbances reduced aboveground biomass and altered plant community composition, but sand dune surface erosion was negligible as belowground ecological functioning was maintained at reduced rates. I propose that the resilience of grass-vegetated sand dune systems to wind erosion and surface destabilization is contingent upon
sustained belowground ecosystem functioning. Understanding the role of belowground functioning in sand dune stability and resilience is critical to identify potential warning indicators and forecast impending regime shifts.

The world’s sand dune landscapes (ergs) have undergone dramatic transitions in the Pleistocene and Holocene (Thomas et al. 2000, Miao et al. 2007b, Yang et al. 2012), switching from stabilized and vegetation covered to mobile, non-vegetated states. The transition of these dynamic landscapes from one state to another is associated with large-scale climate change, where wind patterns and moisture transport are altered (Sridhar et al. 2006b). Globally, these arid and semi-arid landscapes drive the inter-annual variability of the global carbon cycles (Poulter et al. 2014, Ahlstrom et al. 2015), and the state of these landscapes are important as dust emissions from mobilized dunes provide nutrient sources for ocean productivity (Jickells et al. 2005) and affect hydrologic cycles and radiation budgets (Ravi et al. 2011). Although climate is the major driver of the state of dune landscapes, ecological processes such as evapotranspiration and soil respiration mediate climatic impacts. Sand dune stability is affected by vegetation cover, which buffers against wind erosion (Li et al. 2005, 2007), and is viewed as the critical component that determines re-activation or stabilization of sand dunes (Hesse and Simpson 2006). Thus, there are thresholds/tipping points where the resilience to erosion processes is passed (Thomas et al. 2005), and the sand dune system undergoes a critical transition to an alternate, mobilized state that is difficult to reverse (Yizhaq et al. 2009). However, the role of belowground structure and function is relatively ignored compared to aboveground processes, and is likely as important in contributing to the stability of
sand dune surfaces (Bhattachan et al. 2014). High quality roots and soil aggregation facilitate recovery from disturbances (Bardgett et al. 2014, Baer et al. 2015), and decreased soil respiration rates are linked with ecosystem change (Kopittke et al. 2013). Additionally, belowground diversity and function of micro-organisms have a key role in determining ecological responses to environmental change (Bardgett and van der Putten 2014), and plant-soil feedbacks are an increasingly important concept for explaining ecosystem responses to change (Bardgett and van der Putten 2014).

Here I provide results on the responses of above and belowground ecological processes to experimental vegetation disturbances in a stabilized dune field. This study was conducted in the semi-arid sand dune grasslands of the Nebraska Sandhills, the western hemisphere’s largest sand dune field, occupying over 50,000 km² in the central Great Plains. For ten years (2005-2014) vegetation was altered using Pulse and Press disturbances, which provided insight into the stability and resiliency of the sand dune grasslands (Collins et al. 2011). Vegetation was killed and re-growth prevented in the Pulse treatment with herbicide glyphosate (without physical disturbance) in 2005, 2008, 2011, and 2014, but allowed recovery during intervening years. The Press treatment was initiated in 2005 by applying herbicide glyphosate to kill vegetation (without physical disturbance), and re-growth prevented with further herbicide applications. I measured aboveground biomass, soil CO₂ respiration rates, soil N availability (NO₃⁻–N and NH₄⁺–N), net N mineralization, and soil erosion rates.

The experimental Press treatment (2005-2010) examined the ability of sand dune grasslands to provide geomorphic stability, and the time frame of the transition from
intact, grass stabilized sand dunes to mobilized, active sand dunes. Aboveground dead biomass persisted through the first year, but disappeared rapidly in the study’s second year (Figure 6-1). By the study’s third year (2007), the mean aboveground dead biomass was reduced to ~8% of the total dead biomass recorded at the beginning of 2005 (Figure 6-1). The soil CO$_2$ flux, a measure of belowground functioning (Bardgett and van der Putten 2014), persisted longer than aboveground biomass (Figure 6-1), and displayed a slow decline relative to other belowground processes (Figure 6-2). By the third year, when aboveground biomass was largely gone, mean annual soil CO$_2$ flux remained at 39% of the flux rates in the Control (Figure 6-2). By the fifth year (2009), mean annual soil CO$_2$ flux was 18% relative to the Control (Figure 6-2), and soil erosion became apparent (Figure 6-1) as erosion of the top 10cm (A horizon) occurred. Erosion activity in the Press treatment remained consistent with the Control until the fifth year in the study (2009), when rates of soil transport increased above 1cm per three-month period, and in 2010 erosion activity further increased to >10cm per three-month period (Supplemental Figure 6-4). Cumulative erosion varied by topography (Supplemental Figure 6-5), but by the end of the sampling in 2010, an average of 33.72 ± 6.03 cm of sand was lost (Figure 6-1).

Although erosion activity and soil lost was not apparent until the study’s fifth year, the variance in erosion activity increased the year before (2008) significant soil loss occurred (Figure 6-3), which provides a potential warning indicator of the critical transition to a mobilized sand dune state (Scheffer 2010). A key point from the Press treatment is that belowground ecological functioning persisted in the absence of
aboveground vegetation cover and inputs of carbon from vegetation. This contrasts with
dune literature, which generally assumes an aboveground vegetation threshold to prevent
dune activation (10-15% cover; Wiggs et al. 1995, Li et al. 2005). Instead, we see the
belowground legacy of the native C₄ grasses, which allocate the majority of their
production to dense fine roots. These roots decompose slowly (Wedin et al. 1995) and
sustain belowground heterotrophic activity (Wardle et al. 2004). My results show that
persistent vegetation suppression exceeds the resilience of the sand dune ecosystem and
leads to a mobilized sand dune state in 4 – 5 years.

The experimental Pulse treatment examined ecosystem resilience and the ability
of the grassland to recover following severe short-term disturbance. During treatment
years, mean aboveground live biomass at peak production (late summer) was 3 – 49%
relative to the Control (Figure 6-1, Figure 6-2). The pulse-recovery cycles resulted in
large variations (3.8 – 16.9 times greater) in relative forb biomass in recovery years
(Figure 6-2), and an overall decrease in grass (23%) and dead biomass (60%) relative to
the Control (Figure 6-2). Killing the vegetation every three years reduced the mean
growing season soil CO₂ flux by 10-30%, yet inter-annual fluctuations were relatively
dampened compared to other belowground processes (Figure 6-2). Despite the dramatic
shift in plant community composition and the disruption of belowground functioning
(Figure 6-2), the sand dune plots in the Pulse treatment were stable at the end of 2014
(Figure 6-1, Supplemental Figure 6-5). Erosion activity in the Pulse treatment tracked
the Control, with <1 cm of erosion per three-month seasonal period from 2005-2014
(Supplemental Figure 6-4).
However, I did record consistently higher variances in the measured sand movement in the Pulse treatment in late 2014 and early 2015, indicating increased spatial activity in the sand dune surface (Figure 6-3). Sand removal and deposition in the Pulse plots still cancel each other out, but the soil surface is becoming more active, especially on the dune ridges (Figure 6-3), where a blowout (5m by 10m, 30cm deep) occurred in the winter and spring of 2014-2015 (Supplemental Figure 6-6). The increased variance in erosion activity and small blowouts may potentially be foreshadowing a state shift in the Pulse treatment plots (Scheffer 2010). The shift in species composition and vegetation structure will alter root structure, biomass allocation, N cycling, and decomposition rates (Nippert and Knapp 2007, Angelo and Pau 2015), which may alter the resilience of the system and reduce the recovery time from perturbations. It remains to be seen if the current stability of the sand dunes in the Pulse treatment is due to the belowground legacy of the C₄ dominated perennial grassland that existed before treatments began in 2005, or if the resilience of the sand dunes are not dependent on grass composition, as long as there are annual weeds available in the seed bank. I hypothesize that continued periodic disturbances will reduce recovery rates (Veraart et al. 2012) and overcome the resilience of the system, leading to sand dune destabilization.

A key point from this study is that belowground processes affect dune surface stability and resilience. Measuring reduced recovery times, gradual slowing down, and increased variability of belowground ecological processes are important to quantitatively identify thresholds and predict critical transitions (Scheffer 2010). Soil respiration is an ideal measurement in that it reflects the belowground investment of plant carbon, the rate
of decomposition, and it captures responses to abiotic properties such as soil moisture content and temperature. Incorporating measurements of belowground activity such as soil respiration is advantageous because it integrates autotrophic and heterotrophic respiration, providing an indicator of the state and functioning of the system (Oyonarte et al. 2012).

Evidence from this ten-year study indicates that belowground, and not solely aboveground, controls are the basis for grassland sand dune resilience in the Sandhills. Shifts in aboveground plant community composition and structure lead to shifts in the belowground function as indicated by increases in soil $\text{NO}_3^-$, $\text{NH}_4^+$, and reductions in soil $\text{CO}_2$ flux. Aboveground vegetation is not unimportant in this ecosystem, but there is a time lag between disruption of aboveground vegetation, loss of belowground functioning, and sand dune destabilization. The nature and length of this time lag is critical to the stability in this system, and will be dependent on the nature, severity, and duration of disturbances (Scheffer et al. 2009). I propose that the resilience of the grass stabilized sand dunes to wind erosion and surface destabilization is contingent upon sustained belowground ecosystem functioning. These responses may be unique in the context of global dune systems because of the grassland cover and the legacy effect of the C$_4$ grass root systems (Monger et al. 2015). However, the insights of belowground functioning to dune surface stability are still relevant in other dune systems. Understanding the role of belowground functioning in sand dune stability and resilience is critical to identify potential warning indicators and forecast impending regime shifts.
Figure 6-1. Mean (±1 SE) aboveground biomass (g m$^{-2}$; panels A, B, C), soil CO$_2$ respiration (µmol m$^{-2}$ s$^{-1}$; panels D, E, F), and cumulative soil erosion (cm; panels G, H, I) for Press (panels A, D, G), Pulse (panels B, E, H), and Control (panels C, F, I) treatments from 2004-2014. Mean aboveground biomass is not shown for the Control in 2010 because swale locations were underwater, and only ridge locations were available to calculate means. The Press treatment ran from 2005-2010, and arrows denote when Pulse treatments were applied in 2005, 2008, 2011, and 2014.
Figure 6-2. Aboveground and belowground responses in Press (panels A and C) and Pulse treatments (panels B and D), relative to the Control, from 2004-2014. The spline curves (Sigmaplot v13.0) were fit using the relative responses of the mean peak aboveground biomass (total live, live grass, live forb, dead), mean annual growing season soil nitrate concentrations, soil ammonium concentrations, soil net N mineralization, and soil CO2 flux, plotted on a log10 scale. Arrows denote the start of the Press treatment (2005-2010), and the years when the Pulse treatment was applied (2005, 2008, 2011, and 2014).
Figure 6-3. Variance in erosion activity of Control, Pulse, and Press treatments across topographic positions in a sand dune profile, plotted on a log$_{10}$ scale. Erosion activity is the absolute value of seasonal (winter, spring, summer, and fall) soil erosion rates (cm). The Press treatment ran from 2005-2010, and the Pulse treatment was applied in 2005, 2008, 2011, and 2014.
Figure 6-4. Erosion activity for the Control, Pulse, and Press treatment across topographic positions in a sand dune profile. Data points are the absolute values of the mean (±1 SE) erosion rate for a three-month seasonal period, on a log$_{10}$ scale. I calculated erosion rates by determining the amount of soil lost or gained from the previous measurement, which were then summed over a three-month seasonal period (winter, spring, summer, and fall). The Press treatment ran from 2005-2010, and Pulse treatments were applied in 2005, 2008, 2011, and 2014.
Figure 6-5. Cumulative soil erosion (cm) on dune ridges, northeast slopes, southwest slopes, and swales in the A) Press treatment, B) Pulse treatment, and C) Control. Measurements of surface erosion are relative to initial soil surface measurements in June of 2004. Positive values indicate soil deposition, and negative values indicate soil loss. The Press treatment was discontinued at the end of 2010, with re-vegetation efforts from 2011-2015. The Pulse treatment was applied in 2005, 2008, 2011, and 2014.
Figure 6-6. Current state of A) Control and B) Pulse treatments in 2015. A small (5m by 10m, 30cm deep) blowout occurred in the northwest corner of plot 6 (B) in the winter and spring of 2014-2015.
Supplemental Methods

Study Site

This study was conducted within the Grassland Destabilization Experiment (GDEX) located at UNL’s Barta Brothers Ranch (BBR; ~2,200 ha) in the eastern Nebraska Sandhills (42°14′N, 99°39′W; elevation = 765 m) (Wang et al. 2008; Sridhar and Wedin 2009). BBR experiences a typical mid-continental climate located within the transition zone between semiarid and sub-humid climates, and is characterized by periodic droughts and large seasonal and inter-annual variability in rainfall. Mean annual temperature at BBR is 8.1 °C and mean annual precipitation is 576 mm (Wang et al. 2009b), where 80% of precipitation falls during the growing season between April and September (Schacht et al. 2000). During the course of the study from 2004 – 2014, mean annual precipitation was 540 ± 36 mm, with above-average wet years from 2008-2011 and an extreme drought in 2012, where total annual precipitation was 45% (257 mm) of the long-term average. Over the last decade (2004 – 2014), the mean annual air temperature was 8.9 ± 0.3 °C, with eight of the eleven years higher than the long-term average.

In general, around 90% of the BBR Sandhills landscape is composed of upland dunes and dry interdunal areas (swales) dominated by warm-season prairie grasses, while the remaining 10% is naturally subirrigated wet meadows and wetlands. The GDEX site itself is categorized as part of the 90% upland prairie, with no naturally subirrigated wet meadows. The dunes are composed of Holocene eolian sand deposits, which overlie Quaternary and/or Pliocene alluvial sand and silt (Wang et al. 2009a). Across the
Nebraska Sandhills, the dunes are generally oriented from the northwest to the southeast, and dune heights can be as large as 40m high, 825m long, and 1220m wide (Bleed and Flowerday 1998). At BBR, dunes are generally smaller, ranging from 5-20m in height (Wang et al. 2009a) and slopes are 5-15%. The sand dune soils are mostly composed of Valentine fine sand soils with an average of 94% sand and 0.61% OM at 30 cm depth.

This upland mixed-grass prairie community is composed of a mixture of warm and cool season grasses, sedges, forbs, and shrubs. The aboveground net primary production of this mixed-grass community ranges from 100 – 300 g m^{-2} (Wang 2008). Topographic position and aspect across a dune profile affect community composition, with interdunal swale areas (15% of landscape) having a higher proportion of switchgrass (*Panicum virgatum*), bluegrass (*Poa pratensis*), blue grama (*Bouteloua gracilis*), and sedges (*Carex eleocharis*). The dune slopes and ridges (85% of landscape) are composed of warm-season, C_{4} grasses such as prairie sandreed (*Calamovilfa longifolia*) and sand bluestem (*Andropogon hallii*), which are generally located on south facing slopes, while the north slopes contain more cool-season, C_{3} grasses such as needle and thread grass (*Hesperostipa comata*), prairie june grass (*Koeleria macrantha*), and the shrub leadplant (*Amorpha canescens*) (Schacht et al. 2000, Milby 2011). Interdunal swale locations also have greater soil carbon content (0.51%) compared to dune slopes and ridges (0.33%) in the top 30cm, with slightly less sand in the top 10cm of swale locations (91.2% sand) compared to dune slopes and ridges (94.4% sand). Interdunal swale A horizons extended to an average depth of 22.8cm (range 6-41cm), while A horizons on the dune ridges extended to an average depth of 11.8cm (range: 6-21cm; Wang et al. 2008).
Experimental Design

The GDEX project was started in 2004 and was set up with ten circular plots, 120m in diameter (1.1 hectares total area), which span the profile of one sand dune, from swale to dune top to swale. Within each plot, there were 22 subplots, with locations covering the sand dune profile, varying in elevation, aspect, and slope. At the beginning of the GDEX project in 2004, cattle exclosures were constructed around eight of the plots (Plots 1-8), with plots 9 and 10 (Grazed treatment) remaining in the deferred rotational grazing (DRG) studies conducted at Barta Brothers Ranch, where the grazing is moderate and well managed (Schacht et al. 2010, 2011). Two plots were assigned to one of five treatments, which spanned the range of continuous grass cover to bare sand. Treatments included a Control, a Grazed treatment, a Pulse treatment, a Press treatment, and an Aggressive treatment (not used in this analysis). The Control plots (2 and 4), beginning in May 2004 were excluded from grazing, and were not subject to any experimental vegetation or physical soil disturbances. Press and Pulse treatments were chosen for the GDEX project to provide insights into the stability and resilience of the sand dune grasslands (Bengtsson et al. 2003, Collins et al. 2011). Permanent and sustained Press vegetation disturbances demonstrated the time frame required to overcome the resilience of the ecosystem, while short-term Pulse vegetation disturbances (pulse-recovery) showed the ability of the Sandhills grassland ecosystem to recover from periodic, cyclical disturbances. Vegetation in the Press and Aggressive treatments was killed and kept dead with herbicide applications, while vegetation in the Pulse treatment was subjected to
three year pulse-recovery cycles, with vegetation killed with herbicide in one year, and then allowed two years of recovery.

Killing the grass and broadleaf vegetation was done by using applications of Roundup Weathermax (glyphosate 49%), combined with Amine 2,4-D (47%). The initial burn down of the grassland vegetation in the Aggressive, Press, and Pulse treatments was killed using by using a tank mixed combination of glyphosate and 2,4-D, applied at a rate of 5.12 L ha\(^{-1}\) of glyphosate and 5.12 L ha\(^{-1}\) 2,4-D. Subsequent applications of herbicide to suppress vegetation re-growth was applied at lower rates, because the vegetation height and density was reduced compared to the pre-treatment grassland vegetation state. The herbicide reapplications were applied by using a tank mixed combination of glyphosate and 2,4-D, applied at a rate of 1.68 L ha\(^{-1}\) of glyphosate and 1.68 L ha\(^{-1}\) 2,4-D. Herbicide was applied by using an ATV mounted 25-gallon boom sprayer.

the stability, productivity, and hydrology of the Sandhills. Data from the former Press treatment plots used in the new DREX study (2011-2014) are not used in my analyses, as it was not the focus of my research.

The Pulse treatment was on a 3-year cycle, with vegetation killed and re-growth prevented in first year, and then in the second and third year the vegetation was allowed to recover. Herbicide applications in the Pulse treatment occurred in 2005, 2008, 2011, and 2014. To date, there have been three full cycles, with herbicide applications in 2005, 2008, 2011 and two years of recovery between each Pulse herbicide treatment year. A fourth treatment cycle started in 2014, with 2015 and 2016 planned as recovery years.

The Pulse treatment started on 5/19/2005, with an initial application of herbicide (5.12 L ha$^{-1}$) to kill the grassland vegetation in plots 3 and 6. Vegetation re-growth in 2005 was prevented with a further herbicide application (1.68 L ha$^{-1}$) on 5/27/2005, 7/29/2005, and 8/11/2005. Vegetation in this treatment was then allowed to recover in 2006 and 2007. A second Pulse treatment cycle started in 2008, with herbicide applications (1.68 L ha$^{-1}$) on 5/15/2008 and 7/3/2008. Vegetation was then allowed to recover in 2009 and 2010. A third Pulse treatment cycle started in 2011, with a single herbicide application (1.68 L ha$^{-1}$) on 7/21/2011. Vegetation was allowed to recover in 2012 and 2013. A fourth Pulse treatment cycle started in 2014, with herbicide applied (1.68 L ha$^{-1}$) on 6/5/2014, and vegetation allowed to recover 2015 and 2016.
**Sampling Procedures**

**Aboveground Biomass**

Aboveground biomass (ANPP estimate) was sampled several times across the growing season (April-October) from 2005-2014. Although the sampling frequency changed from year to year, varying from every two weeks to once a month, aboveground biomass was sampled at least five times within the growing season for each year. In 2005, biomass was sampled ten times, nine times in 2006, eight times in 2007, six times in 2008 and 2009, five times from 2010-2013, and six times in 2014. Control, Grazed, and Pulse treatments were sampled continuously from 2005-2014, while Press treatments were sampled from 2005 through 2010, when the treatment was discontinued as the plots had become blowing sand dunes.

Aboveground biomass samples were collected from one designated ridge (dune top) and one swale (interdunal) subplot in each plot. On each sampling date, aboveground biomass samples were hand clipped using two 0.25 m² sub-samples in each subplot location. On the next sampling date, biomass was collected from two more 0.25 m² sub-samples adjacent to the previous month’s sub-sample locations. Vegetation was sorted into six categories, roughly based on functional plant groups and photosynthetic components; 1) grass/sedge (monocots), 2) forbs, 3) shrub stems, 4) shrub leaves, 5) cacti, and 6) dead plant material. Biomass samples were then be oven-dried at 55 °C for a period of no less than seven days. All biomass samples were weighed after drying to determine biomass weight.
Extractable Soil N

Extractable soil N and net N mineralization in GDEX was measured over 11 years (2004-2014), with varying numbers of growing season sampling intervals conducted over the course of the project. Sampling generally occurred over the growing season (April-October) with a single winter sampling interval covering the end of the growing season to the next spring (measured from 2004-2009). In 2010 and 2011, sampling was scaled back to a single monthly sampling interval during the growing season due to time, labor, and resources. From 2012-2014 the sampling schedule was expanded back to the full growing season. Along with varying sampling intervals, the treatments and subplots within treatments changed over the 11 years. During the 2004 (pre-treatment) growing season sampling period, four subplots in each of the Control and Press treatment plots were measured for net N mineralization. In 2005, six subplots in each of the Control, Pulse, and Press treatment plots were sampled, with the Press sampling ending after 2010, and the Control and Pulse sampling expanding to 12 subplots from 2012-2014. Subplot locations were chosen to cover the variation in the topographic range across a sand dune profile.

Net N mineralization was estimated monthly, by in situ soil incubation using 1.9 cm diameter plastic tubes (PVC) to a depth of 20 cm, and covered with a cap to prevent leaching losses. Initial soil cores were sampled concurrently near the placement of the incubation tubes. Pre and post incubation soil samples (initial and final) were extracted using a 1M KCl solution, shaken for 0.5h, and then allowed to settle overnight at 4 °C. The settled solution was then pipetted out and sent to the Ecosystems Analysis
Laboratory at the University of Nebraska – Lincoln and analyzed for NH$_4^+$–N and NO$_3^-$–N with a Lachat 8500 Series 2 auto-analyzer. A sub-sample from each soil core was used to determine the gravimetric soil moisture. Net N mineralization (µg N g dry soil$^{-1}$) is the sum of the net in situ ammonification and nitrification and is determined by sum of the final minus the initial values of NH$_4^+$ and NO$_3^-$ for the sampling interval. Growing season and annual net N mineralization was calculated by summing the net N mineralization of all sampling intervals within the growing season (April-October) and through the rest of the year.

**Soil CO$_2$ Flux**

From 2004-2014, the frequency of soil CO$_2$ flux measurements were variable, but over the 11 years there were at least six sampling dates during the growing season (April-October) in each year. From 2005-2009 sampling generally occurred monthly during the dormant season (November-March) and then every two weeks during the growing season. From 2010-2014, dormant season sampling was discontinued, and growing season sampling varied from every two weeks to once a month. Control and Pulse treatments were sampled continuously from 2005-2014. The Press treatment was sampled from 2005 through 2010, and then was discontinued as the plots had become mobile sand dunes and efforts began to re-stabilize the soil. The Aggressive treatment is not used in this analysis.

Soil CO$_2$ (µmol m$^{-2}$ s$^{-1}$) flux was measured monthly using the LI-8100 CO$_2$ flux system (LI-COR, Inc., Lincoln, NE). The LI-8100 was equipped with a type-e thermocouple for soil temperature and a Delta-T theta probe for soil moisture. Each sub-
plot was equipped with a LI-8100 soil collar, constructed from 4” thin walled PVC. LI-8100 soil collars were placed in the southwest quadrant of the sub-plots until approximately 2 cm remains above the soil surface. Soil CO₂ flux measurements were conducted between 0900-1600 hours CST (mean = 1330 hours CST), with values generally reflecting the peak daily soil respiration rate instead of the daily average, which would require continuous flux measurements. When making a measurement with the LI-8100, the thermocouple was inserted into the soil near the collar to a depth of 15 cm and the theta probe was inserted near the collar, but the functional depth was roughly about 7 cm. At each subplot, a single CO₂ flux measurement was determined by measuring the ambient CO₂ within the IRGA and recording observations every 5 seconds for 60 seconds. The first 20 seconds of observations (4) were discarded due to normalization of the CO₂ within the IRGA. The CO₂ flux was then calculated by the LI-8100 using the final eight observations of ambient CO₂. The data file containing observations of CO₂ flux was checked for integrity using the LI-8100 file viewer software, confirming that fluxes were calculated for each sub-plot and correcting for any errors in calculations. Across the four treatments and ten years of sampling, there were 16,811 observations.

**Erosion**

Erosion of the sand dune soil surface was determined at each subplot location (n=22) within each plot, by measuring the distance (cm) from the soil surface to the top of the subplot pin. Subplot pins were installed in each plot in the summer of 2004, and set at a height of 60cm above the soil surface. Subplot pins were located across the sand dune topographic profile within each plot, and were categorized into dune ridge,
northeast slopes, southwest slopes, and interdunal swale locations. Pin height above the soil surface (erosion) was measured every two to four weeks during the growing season (April-October), from 2005-2015. The cumulative amount of soil lost or gained was determined by the soil surface height, relative to the initial starting point in 2004. The erosion rate for any sample period is the difference in the soil surface from one sampling period to the next, where negative values indicate loss of soil, and positive values indicate soil deposition. The erosion activity is the absolute value of the erosion rate, and indicates how much sand was transported (lost or gained), during a sampling period.
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