5-2015

Plant Diversity Affects Performance of Invasive Thistles in Restored Nebraska Grasslands

Katilyn J. Price
University of Nebraska-Lincoln, pricekk11@aol.com

PLANT DIVERSITY AFFECTS PERFORMANCE OF INVASIVE THISTLES

IN RESTORED NEBRASKA GRASSLANDS

by

Katilyn J. Price

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree Master of Science

Major: Natural Resource Sciences

Under the Supervision of Professor David A. Wedin

Lincoln, Nebraska

May, 2015

PLANT DIVERSITY AFFECTS SUCCESS OF INVASIVE THISTLES

IN RESTORED NEBRASKA GRASSLANDS

Katilyn J. Price M.S.

University of Nebraska, 2015

Adviser: David A. Wedin

Invasive plant species threaten native grasslands, affecting nutrient cycling, biodiversity, wildlife habitat, and usable land for production. Consequently, preventing establishment of invasive species is critical before removal becomes difficult and expensive. The purpose of this study was to examine the effects of grassland plant diversity on musk thistle (*Carduus nutans*) and Canada thistle (*Cirsium arvense*) establishment and determine which environmental factors (light penetration, soil moisture, plant diversity, and soil nitrogen) account for resistance to invasions. In a field experiment at The Nature Conservancy's Wood River site (Nebraska), the two invasive thistle species were planted into replicated grassland diversity plots. The 0.3 hectares grassland plots were seeded as monoculture (*Andropogon gerardi*), low diversity, and high diversity treatments in 2010. The experiment also included plots maintained as bare soil. Plant diversity was measured in 2013. Environmental factors were measured during the growing seasons (April-October) of 2013 and 2014. After two years, both thistle species flourished in bare soil plots, maintained populations in monoculture and low diversity plots, while thistles in the high diversity grassland plots emerged but died prior to completing their normal life cycle. Analyses of the environmental factors show strong

declines in resource availability (light, water, nitrogen) associated with both plant biomass and diversity across the experimental diversity gradient.

Acknowledgements

Thank you to the University of Nebraska Foundation (Alan & Irene Williams Endowment Fund) and University of Nebraska (West Central Research and Extension Center) for funding my graduate research assistantship and project.

I would like to thank my co-advisors Dr. Dave Wedin and Dr. Steve Young. I would like to thank Dr. Steve Young for giving me the opportunity to pursue my Master's Degree even though I did not have the strongest background in this area of study. I would like to thank Dr. Dave Wedin for always being willing to meet to help fill in my knowledge gap especially in statistical analysis. Thank you for always having an open door policy to discuss any questions or concerns I had with my experiment and the requirements to graduate. I would like to thank them both for answering my endless questions and offering me advice, encouragement and guidance through this entire process. I would also like to thank them both for being so understanding when I had to take some time off when I became ill and allowing me to come back at my own pace. I would like to thank Dr. Craig Allen for providing support and participating on my committee. I would like to thank Jeremy Hiller and Jeff Hartman for locating and teaching me how to use a variety of field equipment, often on short notice. I would like to thank Kyle Chapo for assisting me in the field and for always taking on any task I needed completed. I would like to thank Alicia Admiral, for helping me to identify all of my plant species as well as answering all of my botany questions in the field. I would like to thank Jerry Volesky and Angie Peterson for helping me run an experiment and collecting data for me in North Platte, NE since I could not always be there. And finally I would

like to thank Chris Helzer and The Nature Conservancy for allowing me to plant two invasive thistles in their beautiful research prairies in Wood River, NE.

I would like to thank my friends and family who always supported me, made me laugh, edited my many papers, and provided me with encouragement during the difficult times. I could not have done it without you.

Table of Contents

List of Tables

List of Figures

Chapter 1:

LITERATURE REVIEW

Katilyn Price

Literature Review

Invasive plant species can alter an ecosystem by outcompeting native plants, reduce native species populations, reduce carrying capacity of the area, alter migration paths, impact human health, modify agriculture, and influence the economy negatively. There are approximately 4,200 species of introduced plants in the United States alone; costing an estimated 138 billion dollars a year (Westbrook 2004). It is important to identify invasive species early on in their establishment or they become more difficult and expensive to remove as their populations expand. Once an invasive species establishes and alters an ecosystems restoration can be difficult. Studying the effects they can have on an ecosystem helps to determine the management programs that can be utilized to combat them.

Not all non-native plants become invasive; approximately 10% of plants have an apparent impact on a given ecosystem (Humle 2013). In order for an invasion to be successful, a plant species must be introduced, establish in the ecosystem, be able to reproduce, and integrate with the other plants in the ecosystem (Booth et al. 2010). An invasive species has to be able to outcompete native plants, adapt the new ecosystem and be extremely plastic to survive outside its native habitats. This impact can range from loss of topsoil, food products, or a loss of aesthetics, recreation, carbon sinks and economic production (Pejchar & Mooney 2009). The impact depends on the location of the ecosystem, the functions it provides, the abundance of the invasive plant and the effect each individual plant can have. Any environmental impacts, nutrient changes, changes to agricultural production, can be quantified and measured to see the effects of the invasive plant. Impacts of invasive species on cultural services, the aspects of an

ecosystem that are non-consumptive (i.e. aesthetics, recreations, tourism), are difficult to assess, because they are based on individual value systems (Pejchar et al. 2009). A lack of quantifiable evidence that a plant is affecting an ecosystem can make assessing the impacts and effects of restoration projects difficult. Understanding the impacts of each invasive species is important in order to protect native habitat, native species, resources and the functions of an ecosystem.

There are three stages an invasive species goes through once it has been dispersed at a site to become a successful established population (Shigesada and Kawasaki 1997). First, there is the initial establishment phase where the species is present in the ecosystem but does not expand its population. Then, there is the invasion phase where the invasive expands and reproduces throughout the area. This phase generally comes after a period of time, which allows for the invasive to maintain its small population and adapt to the new ecosystem. Finally, there is the establishment phase where the invasive species has established in a large population throughout the region, but may be limited by geographical boundaries (Shigesada and Kawasaki 1997). These stages have no specific time limitations, it can take the period of a growing season or years for a species to establish. Invasive species take advantage of an unused area or underutilized resources, or directly outcompetes native species in order to establish and alter the site for their own success. An invasive may be successful for a number of reasons, including propagule pressure, which is the number of subsequent release of a species and the number of individuals per release, which may influence the invasive plant’s establishment success. However, propagule pressure may not be necessary in environments where native species have been removed by disturbance (Theoharides and Dukes 2007). An invasive can

establish in an ecosystem where there is an available niche. It can take advantage of a recent disturbance or opening caused by fire, flooding or overgrazing. If there are no natural enemies to control its population, whether it is insects, herbivores, or pathogens, an invasive may be more successful. Additionally, an invasive species can establish by directly outcompeting a native plant species in a community therefore creating its own space to move into. These factors are not mutually exclusive, as an invasive can become established through one or more of these exploits (see Holzmueller & Jose 2009 review for detailed invasion hypotheses).

For the Great Plains region, the continued spread of invasive species can significantly impact agricultural production, native grasslands, and native animal species that rely on prairies. Native grasslands are important to maintain plant diversity, topsoil, reduce erosion, and habitats for native species. Most of the soil in the Great Plains region is highly fertile and most land has been converted for agricultural purposes. Tallgrass prairies once were located throughout the eastern edge of the Great Plains, but today, only fragmented native patches remain. These areas were historically exposed to disturbances such as fires and grazing by large herd of bison. Now they are maintained through diverse management plans that simulate natural disturbances. These prairies are dominated by big bluestem (*Andropogon* geradii Vitman), switch grass (*Panicum virgatum* L.), and Indiangrass (*Soghastrum nutans* L. (Nash)). Most of their biomass is belowground. These prairie fragments provide the only available habitats for native species, including the greater prairie chicken and black footed ferret. The remaining fragmented patches of native prairies have not been altered, mainly, because the land was not considered useful farming land. In recent years there has been a large effort to restore

native grasslands and promote habitats for these native species, especially ones whose populations are threatened.

In 1985, the United States Government enacted a farm bill that promoted native grassland restoration through the Conservation Reserve Program (CRP). The original goal of the program was to pay farmers to plant native grasses in an effort to prevent soil erosion in marginal lands. The government would provide three to five year contracts that paid the landowners to develop CRP lands on their property. Only in 1990, did the program expand to include wetlands, riparian corridors, and native wildlife habitats. With the continued growth of this program and others, many native habitats have been restored in the Great Plains region. Now, these restored areas are facing a different problem, impactful invasive species. Invasive species, such as musk thistle (*Carduus nutans* L.), leafy spurge (*Euphorbia esula* L.), and purple loosestrife (*Lythrum salicaria* L.), can establish then take over these restored grasslands defeating the entire purpose of the restored areas, further reducing native species. In the Great Plains region, invasives have a huge impact on crop field production and the carry capacity of rangelands. Invasive species like Canada thistle (*Cirsium arvense* L.) are unpalatable to cattle; cattle avoid the invasive plant and the plants surrounding it in favor of other forage grasses. In the case of harvesting wheat, barley or alfalfa, an invasive species in the harvest can make an entire field unsellable, which costs the famer thousands of dollars. If invasives are clumped in a few areas of the field, then crop loss estimates for the entire field will be lower than if they were randomly distributed throughout the field (Booth et al. 2010). If the invasive species are in a patch or a portion of the field, it is possible to harvest around it. However, if it is established throughout the entire field, it can be impossible to avoid a

contaminated harvest. Invasive species can lead to smaller crop and cattle yields overall, which leads, to an increased cost of meat and other agricultural products for the public. It is important to manage invasive species in native grasslands and agricultural lands, otherwise a significant amount of resources can be lost.

Maintaining a healthy, diverse grassland provides a variety of habitats and food sources for the native insects, birds and small mammals of the Great Plains region. Grassland ecosystems with a high diversity of plants are thought to have the most resources utilized leaving little available for introduced species (Davis et al. 2000). Diversity helps to maintain an ecosystem and provide it with vital services, such as nutrient cycling, productivity, and decomposition (Tilman et al. 1996, 2002). Diverse grasslands are considered to be more stable than low diversity or monoculture areas (Tilman and Downing 1994). Management plans need to be varied to permit each species to go to seed over the long term, which prevents the species from dying off. The plans also need to be flexible to accommodate for years when a certain species, may reproduce during a different time, due to varying environmental conditions. Management plans for diverse grasslands need to be flexible by accommodating for environmental conditions each year, controlling undesirable species such as invasives and maintaining the diversity level.

A variety of studies have shown that diverse communities are less susceptible to invasions for a variety of reasons, such as, low nutrient availability or species abundance (Gross et al. 2005, Tilman 1997). Plant communities with high diversity are more resistant to an invasive, because it is likely to interact with a strong native competitor (Pimm 1989). If a community is utilizing resources effectively, there can be fewer weak

points within it which prevents an invasive species from becoming established. However, not all introductions into sites with a low diversity are successful (Simberloff 2013). Thus, the level of diversity in an ecosystem does not explain the success or failure of an invasive, but may help an ecosystem's resistance by reducing accessible resources. Studies have shown increased availability in light, moisture, and soil nutrients assist an invasive species' successful establishment within a community (Theoharides and Dukes 2007; Huenneke et al. 1990; Burke and Grime 1996; Parendes and Jones 2000; Davis and Pelsor 2001). An increase in species diversity in a grassland reduces the available nitrogen in the soil reducing the available resource for newly establishing plants (Oelmann et al. 2007, Tilman et al. 2002). All of these studies show that the more species present in a community, the less likely an invasive species is to become established. However, invasive species are still capable of establishing in highly diverse communities. A popular theory, known as the fluctuating resource hypothesis, states ecosystems with high resource availability, due to a disturbance or low resource utilization, are more susceptible to invasions (Funk and Vitousek 2007). This hypothesis also states that a community's invisibility changes over time, which makes it most susceptible to invasions when there is an increase in resources (Gross et al. 2005). If true, when there is an increase in available light, water, or soil nutrients in any given ecosystem, it is most vulnerable to a possible invasive species establishment. These resources can become available after an area is stressed by overgrazing, drought, a change in a fire regime, predation of a species, or flooding. All in all, the invisibility of a grassland is dependent on a multitude of variables, including available resources, recent disturbances, species diversity, and the effectiveness of an invasive species. While no

ecosystems is impervious to an invasive species, certain factors allow for a community to resist them.

For the purpose of this study musk thistle seeds and Canada thistle rhizomes were introduced into restored, native tallgrass grassland plots. The following sections are in-depth information on the two selected invasive species.

Canada Thistle *Cirsium arvense*:

Canada thistle (*Cirsium arvense)*(Figure 1) is a perennial invasive species found through much of the United States except Texas, Oklahoma, Louisiana, Florida, Georgia, South Carolina, and Hawaii. It is originally from Eurasia and is believed to have been introduced to North America in the late 1700s. Canada thistle can be found in native grasslands, rangelands, cropland, ditches, along roads and recently disturbed places. Unlike other thistles, it is able to infiltrate tilled crop areas. The presence of Canada thistle in agricultural fields can lead to losses in yields as it competes for resources. An established stand of twenty Canada thistle shoots per square meter caused estimated yield losses of 34% in barley, 26% in canola, 36% in winter wheat, and 48% in alfalfa seed (McClay 2002). Canada thistle is unpalatable to cattle and native species, which leads to a reduced carrying capacity of rangelands. Canada thistle reduces forage availability and consumption in pastures, because cattle, typically, will not graze near a Canada thistle infestation (Beck 2008). Once it is able to establish a healthy population in an ecosystem, it is extremely hard to get rid of even with intervention.

Canada thistle is a perennial with spiny leaves and purple/white flowers. It begins as a rosette before branching up, growing anywhere from 0.6 to 1.5 m tall, and ending in

a cluster of flower heads. Canada thistle is often seen growing is large monoculture patches; this can be attributed to its extensive fibrous root system. Horizontal roots spread rapidly, and in a single season, many grow 2.7 to 5.5 m laterally and 1.8 to 2.7 m deep (Wilson 2009). It is able to outcompete native plants for underground root space, helping it to continually spread and reproduce. It typically is most competitive in well-aerated, productive, cool soils, but can grow in soil with a 2% salt content (Beck 2008). The plant is able to reproduce through dioecious flowers and rhizomes, which make it very strong competitor. Shoots normally grow every 5 to 15 cm, as the root leads away from the rosette. Each shoot can become its own viable plant if it is separated from its parent plant. Rhizomes can stay dormant in the soil for up to twenty years (Wilson 2009) Female flower heads can produce 40 to 80 seeds per head with large plants capable of producing at total of 5,000 seeds (Wilson 2009). Approximately 8 to10 days after the flower opens up, the seeds are ready for dispersal, mainly through the wind but also by animals and machinery as well. Being able to reproduce multiple ways helps Canada thistle establish successful stands, taking up vital space and available resources.

Musk Thistle *Carduss nutans*:

Musk thistle (*Carduus nutans*) (Figure 2) is a biennial plant, originally from Eurasia, that is found throughout the United States excluding Maine, Florida, Vermont, Alaska and Hawaii. It was introduced to the U.S. in the mid-1800's and has since spread to most states and covers an estimated 7.7 million acres (WRW 2007). Outside of its native habitat this plant is extremely disruptive to grasslands because it outcompeting native species for resources and reduced agricultural production. Musk thistle can grow

anywhere from sea level to approximately 2,500 m in altitude and requires neutral to acidic soils. It does not grow well in wet, dry or shady conditions (USDA 2005). Musk thistle is normally a biennial plant that has purple flowers with spiny leaves and stems. However, if given the right conditions it can behave like an annual. Mature plants range in height from approximately 0.4 m to 1.8 m with multi-branched stems. Each branch contains a purple terminal flower and a single mature plant is able to produce 100 or more flowers. The plant begins producing seeds 45 to 55 days after bolting during its second year. Musk thistle reproduces only through seeds, as a result each plant can produce hundreds of seeds per flower head. About 7 to 10 days after blooming seed dispersal begins. Once the parent plant has finished gong to seed, its life cycle ends. Most of the seeds land within the vicinity of the parent plant, which leads to a clumped pattern of plants throughout an ecosystem (Beck 2008). Musk thistle can produce up to 20,000 seeds per plant, although only a third of these seeds are considered viable (Beck 2008). Seeds can stay viable for up to ten years in the soil (Roeth et al. 2003). Once a seed is in the seed bank, it can wait years for the right conditions to emerge in an attempt to establish.

As musk thistle establishes in a pasture it can have a huge impact on yield by outcompeting forage plants. Moderate infestations have been shown to reduce pasture yields an average of 23% (OSU 2013). This leads to ranchers to reducing the amount of cattle they can maintain per acre. Musk thistle is extremely noxious in grasslands and rangelands because it is unpalatable to cattle and native species, which reduces the carrying capacity of an area. Infestation size generally increases at a rate of 12-15% yearly, but in some areas the rate has been found to be greater (WRW 2007). In the end,

this costs ranchers money, resources, and grazable land. Once cattle graze in an area, it creates a disturbance in the ecosystem, which provides musk thistle a vulnerable environment to expand into if it is established nearby. Only unfavorable environmental conditions, such as drought, limit the spread of the musk thistle.

Thesis Overview

Invasive plant species threaten native grasslands, which alters nutrient cycling, biodiversity, wildlife habitat, and usable land for production. Consequently, preventing the establishment of invasive species is critical, because future eradication can be expensive and time consuming. The USDA defines an invasive species as a non-native to the ecosystem under consideration and whose introduction causes, or is likely, to cause economic or environmental harm or harm to human health (NISIC 2012). For the purpose of this study, invasive species will be defined according to the ecological definition. A species is considered invasive when it is introduced into a natural or semi-natural habitat, where it alters some part of the community (Simberloff et al. 2012). A successful plant invasion requires that a species is dispersed into an ecosystem, colonizes, establishes, reproduces, spreads and integrates within the community (Booth et al. 2010). In the Great Plains region, the continued spread of invasive species has significant impacts on native grasslands, habitats for native species, cattle production, and crop production. Native grasslands are important for maintaining biodiversity, reducing erosion, providing a carbon sink, and producing forage. Invasive species can establish large patches, alter an ecosystem to favor their spread, and hinder the growth of native species. Certain species are unpalatable to cattle; the cattle avoid the invasive plant and the plants around it in favor for other forage grasses. This study will provide framework to build on previous grassland invasibility studies that endeavor to understand the susceptibility of restored Nebraska native grasslands to invasive musk thistle and Canada thistle by evaluating the available resources. It will lead to better management and restoration practices to help to

reduce the threat of invasive species in grasslands and rangelands in the Great Plains which saves time and financial resources.

The purpose of this study was to examine the effects of grassland plant diversity on musk thistle (*Carduus nutans*) and Canada thistle (*Cirsium arvense*) establishment and determine which environmental factors (light penetration, soil moisture, plant diversity, or soil nitrogen) contribute to the resistance of invasions. The objectives included determining the effects native grasses and forbs at varying levels of diversity had on available soil moisture, light penetration, soil nitrogen, biomass production, and plant cover. The grassland research plots used for this study were planted in 2010 with three different levels of diversity: monoculture, low diversity, and high diversity. Bare ground control plots were created for this study in 2013. The two invasive plant species used were musk thistle and Canada thistle. Musk thistle is a biennial herbaceous plant, with a deep tap root, and reproduces through numerous flower heads during its second year. Canada thistle is a perennial herbaceous plant which reproduces through both flower heads and a large underground network of rhizomes. Both of these thistles have been classified as noxious weeds in Nebraska due to the extensive impacts they can have on a plant community.

I had five main hypotheses that I tested in a field experiment at The Nature Conservancy's research plots in Wood River, Nebraska.

1. The bare ground control plots would have the highest amount of available resources, soil moisture, light penetration, and soil nitrogen, compared to the three plant diversity treatments.

2. There would be more available soil moisture, light penetration, and nitrogen as plant diversity decreased in the prairie plots.
3. As plant diversity increases total live biomass would increase, after two growing seasons.
4. As plant diversity increases the establishment rate and survivorship of musk thistle and Canada thistle would decrease.
5. The grassland diversity plots with the most available resources would be the most susceptible to a musk thistle or Canada thistle invasion.

In Chapter 2, the field experiment at The Nature Conservancy in Wood River, NE will be discussed in-depth. The introduction will address the importance of this study, the knowledge I hoped to gain and the hypotheses. Next the methods and materials section will begin with a site description, a description of musk and Canada thistle, followed by the materials and methods used over the course of the experiment. This will be followed by the results section which is divided into species information, thistle establishment, seasonal trends, and ANOVA results. The discussion section will address overall seasonal and thistle trends seen, the results for each hypotheses, the limitations of the study, and summarize the study. Finally, the chapter will end with a small conclusion section that includes management implications and future studies. Chapter 3 will address a small study on musk thistle establishment response to water limitations in a terrarium experiment at the West Central Research and Extension Center in North Platte, Nebraska.

Literature Cited

Beck, K. G. Musk Thistle. Fact Sheet No 3.102. *Colorado State University Extension*. October, 2008. http://www.ext.colostate.edu/pubs/natres/03102.html.

Booth, Barbara D., Stephen D. Murphy, and Clarence J. Swanton. 2010. *Invasive Plant Ecology in Natural and Agricultural Systems*. CABI.

Burke M.J.W., and J.P Grime. 1996. An experimental study of plant community invasibility. *Ecology* 77: 776–790.

Davis M.A. and M. Pelsor. 2001. Experimental support for a resource-based mechanistic model of invasibility. *Ecology Letters* 4: 421–428.

Davis M.A., P. Grime, and K. Thompson. 2000. Fluctuating resource in plant communities: a general theory of invasibility. *Journal of Ecology* 88:528-534.

Funk, Jennifer L. and Peter M. Vitousek. 2007. Resource-use efficiency and plant invasion in low resource systems. *Nature* Vol. 446 April 2007.

Gross, Katherine L., Gary G. Mittelbach. And Heather L. Reynolds. 2005. Grassland Invasibility and Diversity: Responses to Nutrients, Seed Input, and Disturbance. *Ecology*. 86(2) pp 476-486.

Holzmueller Eric J, and Shibu Jose. 2009. Invasive plant conundrum: What makes the aliens so successful? *Journal of Tropical Agriculture* 47 (1-2): 18-29.

Huenneke L.F., S.P. Hamburg. R. Koide, H.A. Mooney, and P.M Vitousek.1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology* 71: 478–491.

McClay, A.S. 2002. Canada Thistle. *Biological Control of Invasive Plants in the Eastern United States* 217-228.

NISIC. 2012. What is an Invasive Species? *National Invasive Species Information Center***.** http://www.invasivespeciesinfo.gov/whatis.shtml

OSU a.k.a. Oklahoma State University. Musk Thistle. *Oklahoma Invasive Species.* http://oklahomainvasivespecies.okstate.edu/musk_thistle.html

Oelmann, Yvonne, Wolfgang Wilcke, Vicky M. Temperton, Nina Buchmann, Christiane Roscher, Jens Schumacher, Ernst-Detlef Schulze and Wolfgang W. Weisser. 2007. Soil and Plant Nitrogen Pools Related to Plant Diversity in an Experimental Grassland. *Soil Science Society of America Journal* 71 (3):720-729.

Parendes L.A. and J.A. Jones. 2000. Role of light availability and dispersal in exotic plant invasion along roads and streams in the H. J. Andrews Experimental Forest, Oregon. *Conservation Biology* 14: 64–75.

Pejchar, L., and H.A. Mooney. 2009. Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution* 24(9):497-504.

Pimm, Stuart L. 1989. *Theories of Predicting Success and Impact of Introduced Species Biological Invasions: a Global Perspective.* John Wiley & Sons Ltd

Remaley, Tom. 2009. Musk Thistle Fact Sheet. *Plant Conservation Alliance Alien Plant Working Group.*

Roeth, Fred, Steven R. Melvin and Irvin L. Schleufer. 2003. Noxious Weeds of Nebraska: Musk Thistle. *University of Nebraska- Lincoln Digital Commons*.

Simberloff, Daniel. 2013. *Invasive Species: What Everyone Needs to Know.* Oxford University Press. New York, New York.

Simberloff, Daniel, Lara Souza, Martin A. Nunez, M. Noelia Barrios-Garcia and Windy Bunn. 2012. The natives are restless, but not often and mostly when disturbed. *Ecology*. 93(3) 598-607.

Shigesada, Nanako and Kohkichi Kawasaki. 1997. *Biological Invasions: Theory and Practice.* Oxford University Press. New York, New York.

Theoharides, Kathleen A. and Jeffery S. Dukes. 2007. Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist*. 176:256-273.

Tilman, David. 1997. Community invisibility, recruitment limitation and grassland biodiversity. *Ecology* 78(1) pp.81-92.

Tilman, D, .J. Knops, D. Wedin and P. Reich. 2002. Plant diversity and composition: effects on productivity and nutrient dynamics of experimental grasslands. Pages 21-35 in M. Loreau, S. Naeem, and P. Inchausti (eds.). *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives.* Oxford University Press.

Tilman, D., D. Wedin and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*. 379:718-720.

Tilman, D. and J.A. Downing. 1994. Biodiversity and stability in grasslands. *Nature.* 367:363-365.

USDA, 2005. Weed of the Week: Musk Thistle. *Forestry Service Forest Health Staff.* http://na.fs.fed.us/fhp/invasive_plants/weeds/musk-thistle.pdf

Westbrooks, RG. 2004. New approaches for early detection and rapid response to invasive plants in the United States. *Weed Technology* 18:1468-1471.

Wilson, Roberts. 2009. Noxious weeds of Nebraska: Canada thistle. *University of Nebraska-Lincoln Extension* EC171:1-8.

WRW a.k.a. Western Rangeland Weeds. 2007. Economic and Ecological Impacts. Western Rangeland Weeds. http://forestandrange.org/Rangeland%20Weeds%20module/sub3/p5.shtml.

Figures

Figure 1: Canada thistle, *Cirsium arvense,* in early phase located in a control plot at The Nature Conservancy's research plots in Wood River, Nebraska.

Figure 2: Musk thistle, *Carduus nutans,* in early reproductive phase located in a control plot at The Nature Conservancy's research plots in Wood River, Nebraska.

Chapter Two:

PLANT DIVERSITY AFFECTS PERFORMANCE OF INVASIVE THISTLES IN RESTORED NEBRASKA GRASSLANDS

Katilyn Price

Introduction

Invasive plant species threaten native grasslands, which alters nutrient cycling, biodiversity, wildlife habitat, and usable land for production. Consequently, preventing the establishment of invasive species is critical, because future eradication can be expensive and time consuming. The USDA defines an invasive species as a non-native to the ecosystem under consideration and whose introduction causes, or is likely, to cause economic or environmental harm or harm to human health (NISIC 2012). For the purpose of this study, invasive species will be defined according to the ecological definition. A species is considered invasive when it is introduced into a natural or semi-natural habitat, where it alters some part of the community (Simberloff et al. 2012). In the Great Plains region, the continued spread of invasive species has significant impacts on native grasslands, habitats for native species, cattle production, and crop production. Native grasslands are important for maintaining biodiversity, reducing erosion, providing a carbon sink, and producing forage. Invasive species can establish large patches, alter an ecosystem to favor their spread, and hinder the growth of native species (Holzmueller & Jose 2009). Certain species, such as musk thistle, are unpalatable to cattle; the cattle avoid the invasive plant and the plants around it in favor for other forage grasses (Roeth et al. 2003). This study will provide framework to build on previous studies that endeavor to for understand the susceptibility of restored Nebraska native grasslands to invasive musk thistle and Canada thistle by evaluating the available resources. It will lead to better management and restoration practices to help to reduce the threat of invasive species in grasslands and rangelands in the Great Plains which saves time and financial resources.

For the Great Plains region, the continued spread of invasive species significantly impacts agricultural production, native grasslands, and native animal species that rely on the prairies. Tallgrass prairies once were located throughout the eastern edge of the Great Plains, but today, only fragmented native patches remain. These prairies are dominated by big bluestem (*Andropogon* geradii Vitman), switch grass (*Panicum virgatum* L.), and Indiangrass (*Soghastrum nutans* L. (Nash)). Maintaining a healthy, diverse grassland provides a variety of habitats and food sources for the native insects, birds and small mammals of the Great Plains region. Grassland ecosystems with a high diversity of plants are thought to have the most resources utilized leaving little available for introduced species (Davis et al. 2000). Diversity helps to maintain an ecosystem and provide it with vital services, such as nutrient cycling, productivity, and decomposition (Tilman et al. 1996, 2002). Management plans need to be varied to permit each species to go to seed over the long term, which prevents the species from dying off. The plans also need to be flexible to accommodate for years when a certain species, may reproduce during a different time, due to varying environmental conditions. Management plans for diverse grasslands need to be flexible by accommodating for environmental conditions each year, controlling undesirable species such as invasives and maintaining the diversity level.

Invasive species take advantage of an unused area or underutilized resources, or directly outcompetes native species in order to establish and alter the site for their own success. In order for an invasion to be successful, a plant species must be introduced, establish in the ecosystem, be able to reproduce, integrate with the other plants in the ecosystem (Booth et al. 2010). An invasive may be successful for a number of reasons, including propagule pressure, which is the number of subsequent release of a species and

the number of individuals per release, which may influence the invasive plant's establishment success. However, propagule pressure may not be utilized in environments where native species have been removed by disturbance (Theoharides and Dukes 2007). An invasive can establish in an ecosystem where there is an available niche. It can take advantage of a recent disturbance or opening caused by fire, flooding or overgrazing. If there are no natural enemies to control its population, whether it is insects, herbivores, or pathogens an invasive may be more successful. Additionally, an invasive species can establish by directly outcompeting a native plant species in a community therefore creating its own space to move into. These factors are not mutually exclusive, as an invasive can become established through one or more of these exploits (See Holzmueller & Jose 2009 Review for detailed invasion hypotheses).

A variety of studies have shown that diverse communities are less susceptible to invasions for a variety of reasons, such as, low nutrient availability or species abundance (Gross et al. 2005, Tilman 1997). Plant communities with high diversity are more resistant to an invasive, because it is likely to meet a strong native competitor (Pimm 1989). If a community is utilizing resources effectively, there can be fewer weak points within it which prevents an invasive species from becoming established. However, not all introductions into sites with a low diversity are successful (Simberloff 2013). Thus, the level of diversity in an ecosystem does not explain the success or failure of an invasive, but may help an ecosystem's resistance by reducing accessible resources (Gross et al. 2005). Studies have shown increased availability in light, moisture, and soil nutrients have been shown to assist an invasive species successful establishment within a community (Theoharides and Dukes 2007; Huenneke et al. 1990; Burke and Grime 1996;

Parendes and Jones 2000; Davis and Pelsor 2001). An experiment in plant diversity has shown an increase in species diversity in a grassland reduces the available nitrogen in the soil reducing the available resource for newly establishing plants (Oelmann et al. 2007). All of these studies show that the more species present in a community, the less likely an invasive species is to become established. However, invasive species are still capable of establishing in highly diverse communities. A popular theory, known as the fluctuating resource hypothesis, states ecosystems with high resource availability, due to a disturbance or low resource utilization, are more susceptible to invasions (Funk and Vitousek 2007). This hypothesis also states that a community's invisibility changes over time, which makes it most susceptible to invasions when there is an increase in resources (Gross et al. 2005). If true, when there is an increase in available light, water, or soil nutrients in any given ecosystem, it is most vulnerable to a possible invasive species establishment. These resources can become available after an area is stressed by overgrazing, drought, a change in a fire regime, predation of a species, or flooding. All in all, the invisibility of a grassland is dependent on a multitude of variables, including available resources, recent disturbances, species diversity, and the effectiveness of an invasive species (Tilman 1997, Gross et al. 2005, Holzmueller & Jose 2009). While no ecosystems is impervious to an invasive species, certain factors allow for a community to resist them.

The purpose of this study was to examine the effects of grassland plant diversity on musk thistle (*Carduus nutans*) and Canada thistle (*Cirsium arvense*) establishment and determine which environmental factors (light penetration, soil moisture, plant diversity, or soil nitrogen) contribute to the resistance of invasions. The objectives included

determining the effects native grasses and forbs at varying levels of diversity had on available soil moisture, light penetration, soil nitrogen, biomass production, and plant cover. The grassland research plots used for this study were planted with three different levels of diversity: monoculture, low diversity, and high diversity in 2010. Bare ground control plots were created for this study in 2013. The two invasive plant species used were musk thistle and Canada thistle. Musk thistle is a biennial herbaceous plant, with a deep tap root, and reproduces through numerous flower heads during its second year. Canada thistles is a perennial herbaceous plant which reproduces through both flower heads and a large underground network of rhizomes. Both of these thistles have been classified as a noxious weed in Nebraska due to the extensive impacts they can have on a plant I had five main hypotheses that I tested in a field experiment at The Nature Conservancy's research plots in Wood River, Nebraska.

1. The bare ground control plots would have the highest amount of available resources, soil moisture, light penetration, and soil nitrogen, compared to the three plant diversity treatments.
2. There would be more available soil moisture, light penetration, and nitrogen as plant diversity decreased in the prairie plots.
3. As plant diversity increases total live biomass would increase, after two growing seasons.
4. As plant diversity increases the establishment rate and survivorship of musk thistle and Canada thistle would decrease.
5. The grassland diversity plots with the most available resources would be the most susceptible to a musk thistle or Canada thistle invasion.

Understanding the mechanisms of how a community is susceptible or its ability to resist invasive species can lead to better prevention and management plans for musk thistle and Canada thistle.

Methods and Materials

Study Site

The experiment was conducted at the tallgrass prairie research plots located on The Nature Conservancy property just outside of Wood River, Nebraska on West Denman Road (40°44'37.8"N 98°35'23.9"W) . The research plots are divided into four replicates of three different diversity treatments, which include four high diversity, four low diversity, and four monoculture plots (Figure 4). The plots are each approximately 0.3 hectares (60 m x 60 m), with wide alleys in-between the individual plots. The alleys are mowed regularly to maintain the borders of the grassland plots. This area has been developed by The Nature Conservancy to study planting techniques, insect and plant diversity, invasive species, and the habits of some prairie species. The research plots are studied in partnership with several Midwest universities to study a variety of grassland projects. The site consists mainly of loamy soils (Figure 3). Data collected from 1980 to 2010 by NOAA indicates this region has an average annual high of 16.87 ˚C, an average annual low of 3.36 ˚C, and an average annual temperature of 10.1 ˚C. The site had an average annual precipitation of 67.74 cm and snowfall total of 64.54 cm.

The field was previously used to grow corn and it was disced in preparation for planting the research plots. The monoculture plots were planted in February of 2010, while the high diversity and low diversity plots were planted in March of 2010. The monoculture plots were only planted with big bluestem (*Andropogon gerardii* Vitman), the low diversity plots were planted with a mix of local native grasses, and the high diversity plots were planted with the same mix of grasses as the low diversity plots, as

well as, a combination of forbs. Due to the method of planting, there is no available data on the actual seeding rate for this site. In March of 2013, the research plots were burned for the first time.

Invasive Species

Two, 1.8m by 4.3m, subplots were established in each of the twelve research plots. The subplots were treated as independent experimental units in this study. Each subplot had 32 points spaced 0.6m apart where musk thistle and Canada thistle were planted. The grid formation was chosen so that locating the invasive thistles would be easy early in their development and later when the prairie plots became dense. April 15, 2013, all musk thistle subplots were planted with seeds sourced from a previous project at the West Central Extension and Research Center in North Platte, Nebraska. Seed heads were harvested, dried, and stored from 2011 to 2012. Based on a previous germination study and a small germination study in the lab, only 30% of the seeds were viable. In order to compensate for this, three seeds were planted at each point. May 7, 2013 and May 13, 2013. All Canada thistle subplots were planted with rhizomes, each approximately 7.6 cm, sourced from an established stand in North Platte, Nebraska. The rhizomes chosen were healthy looking and had one or more buds on them. All rhizomes were considered viable, so only one clipping was planted per point.

Control Subplots

Eight bare ground control subplots were created on the western side of the large prairie research plots. This area had not been seeded with native species and was part of the area that was mowed to maintain the plots. April 29, 2013, the control subplots were

sprayed with Roundup to kill of all plants to create bare ground plots. On May 7, 2013, the control plots were planted with musk thistle and Canada thistle. During the two growing seasons the control subplots were weeded biweekly for any species other than the invasive thistle. Additional herbicides were not used to maintain the bare ground subplots because of the possible risk to the healthy thistles.

Subplot Measurements

The following readings were taken bi-weekly over two growing seasons: light penetration (Decagon Accupar LP-80), soil moisture to 5 cm depth (Delta-T Devices SM 200), plant cover (Daubenmire 0.5 m^2 quadrats), and thistle density/size. Soil nitrogen (KCl-extractable nitrate and ammonium) was measured from soil samples collected three times in 2013 and 2014. Each plot was surveyed for plant species composition in 2013. All data was written in the field and entered in excel for analysis later. This data will provide the framework for understanding what environmental factor(s) are available at each level of diversity and which would allow for the establishment of an invasive species.

Light Penetration

Above and below canopy photosynthetically active radiation (PAR) readings were taken, the percent light penetration (TAU) was then calculated for each subplot using a Decagon Accupar PAR-80 ceptometer in 2013 and a LP-80 model in 2014. Eight individual readings were calculated per subplot to determine the above and below canopy PAR and TAU. The readings were taken in approximately the same spot each time and were equally spaced around the edge of the subplots facing inwards. All measurements

were made within two hours of solar noon. Readings were not taken on days that were windy or where patchy clouds would cause interference. On overcast days, two above canopy readings were taken to make sure the PAR stayed the same. Eleven points of data were removed from the final analysis, because the TAU was suspect, possibly due to user error or data entry error. This accounts for <0.25% of all the measurements taken.

Soil Moisture

Eight soil moisture readings were taken at a depth of 5 cm using a Delta-T Device SM 200 Soil Moisture Sensor and a HH2 Moisture Meter. The sensor determines the volumetric soil moisture content with a ±3% accuracy. Two readings were randomly taken near grasses, forbs, invasive thistles, and bare ground within the subplot. The bare ground locations had to be 7.6cm or greater in diameter to be considered. Before the invasive thistle seedling emerged, the soil moisture readings were taken at the approximate plant locations. If the subplot did not have any thistles or only one, then additional soil moisture readings were taken near grasses. Soil moisture data was never collected within 24 hours of rain to prevent biased readings. The average soil moisture content for each plot was determined during data analysis.

Plant Cover

The Daubenmire method using a 0.5 m^2 quadrat was applied to collect canopy cover data for each subplot. Four readings were taken bi-weekly in the same location within each subplot. Percent cover was determined for the following functional groups; grasses, forbs, invasive thistle, bare ground, and debris. The plant had to be rooted within

the quadrat to be included in the data. All cover percentages add up to 100% per quadrat and were assigned one of Daubenmire's six cover classes during data entry.

Thistle Density and Performance

The number of thistles per subplot were counted bi-weekly. The thistles were planted in a uniform grid so it was relatively easy to determine the locations of them. In some cases, musk thistles had two small rosettes at a single planting point, to accommodate this special case they were counted as two separate plants. Additional shoots surrounding a Canada thistle parent plant were not included in the counts. Based on thistle census data I calculated two measurements to represent thistle performance. Establishment was the average number of thistles found across the subplots for each treatment in July, 2013. The percent surviving thistles was determined by taking the ratio of observed thistles from July 2013 to September 2014. In a few cases the ratio was greater than 1 when more thistles were found in 2014 than 2013. Additionally, the diameter of each rosette was measured from the tips of the largest green leaves. Once the thistle bolted, the height of the plant was also measured (data not shown).

Nitrogen Sampling

Three, 10 cm, soil cores were randomly collected from each subplot three times over each growing season. Soil samples were never collected within 48 hours of a rain event to prevent biased results. The three soil cores were homogenized in the lab and processed within 24 hours of collection. An approximate 50 ml solution of 1 mol/L KCL was added to a sampling cup and weighed. Approximately 20 grams of soil were then added to the KCL solution, reweighed, mixed on a shaker table, and stored in the fridge

in order to allow the soil to settle. A number of blank KCL solutions were processed each time to determine if the samples were contaminated with outside nitrates and ammonia. After 24 hours, when the soil had settled in the KCL solution, 15 ml of the solution was placed in a small vial and frozen to be processed by the Ecosystems Analysis Laboratory in the School of Biological Sciences at the University of Nebraska-Lincoln. The lab processed each vial for the ammonium, NH_4^+, and the nitrate, NO_3^{--}, content for each subplot's soil sample. The water content, for each subplot's soil sample, was determined by weighing a sampling cup half-filled with soil then letting it dry for 48 hours at 15.5° C and then reweighing the sample.

Species Lists per Plot

The species lists for each whole plot was extrapolated from a floristic quality index done at the site. In July 2013, with the assistance of a botanist, Alicia Admiral, species were identified within each of the research plots. Transects were set up evenly dividing the 60 meters into three, 20 meter-wide sections facing north to south in each of the plots. Five nested 1 m^2 quadrats were evenly placed in each of the divided transects, resulting in 15 replications per plot. The nested quadrat contained the following sizes: the smallest quadrat 1/100 m^2 (1), the medium size 1/10 m^2 (2), and the largest size 1 m^2 (3). Once the quadrat was placed all the plant species were listed indicating the section of the nested quadrat it was located in. Only species rooted within the quadrats placed in the treatment plots were recorded. If a species was noticed in the plot but did not show up in any of the quadrats it was listed separately as well. Species lists were determined for each of the twelve research plots, for the three diversity types overall and finally for the site overall.

Species List per Subplot

A species list for each subplot was determined in July 2013, with the assistance of a botanist, Alicia Admiral. Using the same four replications per subplot used for plant cover, a 0.5 m^2 quadrat was placed. All species rooted with the quadrat were recorded, and given a class cover based on the Daubenmire method. The species list for each of the four replicates were cross-referenced to create a species list for the subplot.

Species Diversity per Subplot

Using the species list created for each subplot, the class cover was converted to the approximate midpoint of the cover values based off of the Daubinmire method. Then the mean species richness was calculated for the subplot by using the 4 quadrats (½m^2), then recalculated into a proportional (relative) abundance. This was then standardized so all individual species cover values in the subplot summed to 1 (i.e. 100%). The Shannon index ($H= - \sum pi(ln)p$) was calculated for across all species within the plot and finally the index was converted to the effective species richness, e^H.

Aboveground Biomass Collection

At the end of the second growing season, August 2014, biomass was collected in each of the subplots. Two, 1/8 m^2, quadrats were randomly placed with the subplot and the vegetation clipped to the crown. In the lab the vegetative matter was sorted into the following functional groups; grasses, forbs, shrub, and dead. Once sorted they were placed in a drying oven at 15.5° C. After a week, the dried plant material was removed and weighed.

Data Analysis

The data for light, soil moisture, biomass, nitrate, ammonia, and number of thistles were all graphed to exam overall trends for each growing season. The site was analyzed as a completely randomized design for all data collected. Two way ANOVAs, with the interaction included, were completed for light, soil moisture, and nitrogen. Followed by Tukey Honest Significant Difference multiple comparison test between the three diversity types (high, low and monoculture) and the bare ground control plots. A step wise regression model was analyzed to determine which resource had the most significant impact on the number of established thistle. A P- value of 0.05 was used to determine statistical difference for all tests. All data analysis was completed using JMP Statistical Software by SAS.

Results

Vegetation in Experimental Grassland Plots

The mean species richness was not significantly different between the two subplots of Canada thistle (CT) and musk thistle (MT) for each of the three treatment types (P>.05) (Figure 5A). Due to this result, the analysis of resource availability for light, soil moisture and nitrogen were not separated by thistle type within the treatments types. There were 55 species located in the high diversity subplots, 40 species located in the low diversity subplots, and 34 species located in the monoculture subplots (Table 1). In the monoculture plots, *Andropogon gerardii* had the highest average percent coverage. In the low diversity plots, *Sorghastrum nutans* had the highest average percent coverage. In the high diversity plots, *Elymus Canadensis* had the highest average percent coverage. Many of the same species were found in each treatment type, however, the average percent cover varied. The mean effective species richness was calculated for the thistle subplots per each treatment to evaluate species diversity (Figure 5B). The high diversity treatments did have the highest level of diversity followed by the low diversity treatment and then the monoculture treatment. The plant composition shows in the first year, after the burn, there is a lot of bare ground present throughout all of the plots as the prairie is recovering (Figure 6). The debris present in the bare ground plots for 2013, were remnants of dead plant material after the herbicide application. The mean cover of forbs stayed consistent for each diversity type from 2013 to 2014. The grasses increased in all of the diversity plots from 2013 to 2014.

Thistle Establishment and Initial Resource Availability

At the earliest phase of establishment (July 2013), there were more thistles growing in the bare ground plots than the three types of diversity plots (Figure 7A). Musk thistle had the most thistles present in the bare ground plots, followed by the monoculture plots, then the low diversity plots, and finally, the high diversity plots. Musk thistle also established better than the Canada thistle, having a higher population in the different plot types. The establishment rate for Canada thistle is not as clear. While the bare ground plots do have the most thistles present, followed by the monoculture plots, the low and high diversity plots had a similar number of thistles present. During the establishment phase of musk thistle and Canada thistle in the bare ground, monoculture, low and high diversity plots, patterns emerged that could explain the higher thistle counts in the bare ground plots compared to the high diversity plots. As the average soil moisture, average light penetration, and average available soil nitrogen increased, the musk thistle and Canada thistle were more likely to successfully establish, during April through June 2013 (Figures 8, 9, 10). A multiple regression analysis of the three resources during the establishment phase had a P-value of .0059, however none of the resources alone were significant. A step wise regression analysis was run to determine the best predictor of thistle success. It suggests soil nitrogen is the most important of the three aspects of resource availability measured, during the establishment phase for both types of thistles. The P-value =0 .038 for NO_3^{--} + NH_4^{+}, while light penetration, soil moisture, and thistle type all had P-values >0.05.

Thistle Performance Over Two Seasons

All plots had established musk thistle and Canada thistle seedlings in July 2013 (Figure 7A). By September 2014, there were no surviving thistles in the high diversity plots (Figure 7B). The low diversity plots, each had reduced musk thistle populations and no Canada thistle populations. The monoculture plots maintained both thistles, however, at reduced populations in 2014. The bare ground control plots only had a few thistles die off between 2013 and 2014. Musk thistle plants produced flower heads (Figure 19) from June to August 2014, with the peak production occurring in July. Control plot 2 produced a total of 482 flower heads, control plot 4 produced 316, control plot 5 produced 440 and control plot 8 produced 125. Canada thistle bare ground control plots produced less flower heads than the musk thistles (Figure 20). There was no peak production for all of the plots on one day, each plot varied. Control plot 1 produced a total of 53 flower heads, control plot 3 produced 5, control plot 6 produced 106 and control plot 7 produced 70. During the first growing season the average thistle rosette size for both species were relatively small and did not differ greatly by diversity type (Figure 21). However, during the second growing season the musk thistle average size peaked in early June and slowly decreased in size as plants senesced in the control plots. The grassland plot rosettes peaked during July. The Canada thistle average rosette size peaked at the end of June for all diversity types. Overall the control plots had the largest thistle rosette size followed by the monoculture plots, low diversity and then the high diversity plots. In the second season, 2014, all of the musk thistles completed their life cycle, however, being perennial the Canada thistle did not (Figure 11). Once the Canada thistle displayed signs of going dormant, they were treated with an herbicide application at the completion of the project.

Resource Availability Over Two Seasons

The average soil moisture, at a 5 cm depth, was more varied for the bare ground control plots, but this observation can be explained by the lack of vegetation in the plots (Figure 12). For the three other diversity types, the pattern was relatively similar with the high diversity plots having the most available soil moisture, followed by the low diversity plots and monoculture plots respectively. The average available NH_4^+ (ppm) in the soil was fairly consistent for both growing seasons (2013, 2014) with the exception of one peak in the monoculture plots at the beginning of 2014 (Figure 13). The cause of this spike is unknown. The average NH_4^+ levels can be seen cycling with the largest available amount in early June of both years, then slowly decreasing through the rest of the season. The average available NO_3^{--} (ppm) in the soil was low for the monoculture, low, and high diversity plots, however, there was significantly more available in the bare ground control plots over both years (2013, 2014) (Figure 14). The average available soil nitrogen (ppm) over both growing seasons shows there was a larger statistically significant ($P<0.0001$) amount of available NO_3^{--} in the bare ground plots than the other plots (Figure 15). The monoculture, low, and high diversity plots each had similar amounts of available NO_3^{--} ($P>0.05$). The available NH_4^+ was not statistically different in the plots ($P>0.05$).

The average percent light penetration over both growing seasons shows a significant trend by treatment type with bare ground having the most, followed by high diversity, low diversity, and monoculture plots (Figure 16). In March 2013, all of the prairie plots were burned as a part of an ongoing management plan, therefore, all of the treatments, bare ground, monoculture, high, and low diversity, began with no vegetative cover and equal light penetration (Figure 17). Throughout both growing seasons, the bare

ground control plots maintained the highest average amount of light penetration, followed by high diversity, low diversity and monoculture plots respectively. Total live biomass was not significantly different (P= 0.09) for each of the diversity types (Figure 18). The biomass for grasses, shrubs, and dead were comparable (P>0.05) for all three diversity types, however, high diversity forbs were significantly different (P= 0.0003) from low diversity and monoculture. Productivity (g/m^2) at the Wood River, NE site is similar to other tallgrass prairies production in the region.

Two-Way ANOVA Results

The ANOVAs were completed in order to evaluate relative importance of sampling date and treatment on resources availability for light penetration, soil moisture and nitrogen. For each of the three resources I performed a two way ANOVA for each of these resources individually, these results were reported in Table 2. The average percent light penetration during the two growing seasons (mid-June to September 2013 and 2014) resulted in a highly statistically significant difference by treatment types and dates (Table 2) The ANOVA showed the treatment difference was highly consistent across the dates. While the treatment by date interaction was significant, it was minor. A Tukey Honestly Significant Difference (HSD) pairwise comparison showed that each diversity type was different from one another. The bare ground plots had the most available light compared to all of the other plots with a mean of 63%. It was followed by the high diversity with a mean of 31%, then low diversity with a mean of 26%, and finally, the monoculture plots with a mean of 23%. The average shallow soil moisture over the two growing seasons, did not have a strong significant difference when comparing the diversity treatments. After doing a Tukey HSD pairwise comparison, the significant result can be explained by

the variability in the bare ground plots, while the other diversity plots were comparable ($P>0.05$). The bare ground plots were only statistically significant from low diversity plots ($P<0.05$). The average soil moisture for the bare ground plots was 20.9%, followed by the high diversity plots with a mean of 20.5%, then the monoculture plots with a mean of 20.3%, and finally, the low diversity plots with a mean of 19.9%. The date was highly statistically significant, which shows the effect the weather had on soil moisture. Therefore, the change in the soil moisture throughout the two seasons cannot be explained by the plant diversity types, rather, it can be seen as an effect of the weather. For the nitrogen two-way ANOVA, the natural Log of the total of NO_3^{--} + NH_4^{+} was used. The Tukey HSD pairwise comparison showed the bare ground control plots were significantly different from the prairie plots ($P< 0.05$), however, the high diversity, low diversity, and monoculture plots were not significantly different from each other ($P> 0.05$). The average available nitrogen for the bare ground plots was 0.76 ppm, followed by the monoculture plots with a mean of 0.33 ppm, then the low diversity plots with a mean of 0.3 ppm, and finally, the high diversity plots with a mean 0.21 ppm.

Discussion

During the 2013 growing season, musk thistle and Canada thistle established in all of the twelve subplots in each of the diversity treatments. The populations for both thistles peaked in July 2013, and declined in the grassland plots for the remainder of the season. All of the musk thistles and Canada thistles stayed in rosette form during the first growing season. Over-winter mortality occurred for some of the rosettes in all of the diversity plots, however, fewer thistles died in the control plots than the prairie plots. In 2014, the thistle population peaked in June in all plots, but slowly declined in grassland diversity plots. All in all, the musk thistle established and survived better than the Canada thistle in all of the plots. At the end of the second growing season, thistles only persisted in the low diversity and monoculture prairie stands, indicating a stronger resistance to thistle survival in the high diversity plots.

Of the five main hypotheses, data collected supported hypotheses 1, 3, 4 and only partially supported 2, and 5. The bare ground control plots had the highest available resources (light, soil moisture, and soil nitrogen) over both growing seasons, therefore, the most successful musk and Canada thistles (hypothesis 1). The high availability of all of these resources can be attributed to the lack of vegetation present in these plots. The control plots were the only plots to have flowering musk and Canada thistles. With the exception of one monoculture musk thistle, they were the only plots to have bolting musk thistles. There were a few Canada thistle plants in the monoculture plots that bolted, but they never produced flowers. The Canada thistles in the control plots not only produced flowers, but also spread via rhizomes, which was not seen in any other plot. All in all, the

musk thistle and Canada thistle thrived in the bare ground plots due to the higher availability of the following resources, light, soil moisture and soil nitrogen.

During the establishment phase (June to July, 2013), musk thistle and Canada thistle were more likely to establish in plots with high soil moisture, light penetration, and available soil nitrogen. Based on this criteria and observations in the field, the bare ground control plots had the most thistles established. The monoculture plots had the highest available soil moisture, soil nitrogen, and light penetration. Which contributed to them having the most musk thistle and Canada thistle establish followed by the low and then the high diversity. The multiple regression analysis of all of the available resources, measured during the establishment phase, suggested the available soil nitrogen was the most important of all of the resources studied. Since the prairie plots had just been burned in March 2013 as part of an ongoing management plan, resources were more readily available, as the prairie was growing back in the early spring. Therefore, the resources available during the early prairie regrowth and thistle establishment changed during the remainder of the season, as the prairie reestablished. This could have contributed to the success rate of the establishment of musk thistle and Canada thistles.

Originally, I had hypothesized there would be more available soil moisture, percent light penetration, and soil nitrogen in the grassland plots as plant diversity decreased (hypothesis 2). This was true for the soil nitrogen, as there was less available in the high diversity plots when compared to the low diversity and monoculture plots, respectively. However, as diversity decreased, there was less available light penetration in the prairie plots. This can be attributed to the higher amounts of grass cover present in the low diversity and monoculture plots, as the grass stands were much denser than the

forbs. The available percent light penetration did not have an impact on the growth of musk and Canada thistle as the high diversity plots, which had the most light availability, had no thistles present. While monoculture plots, with the least amount of light availability, had the highest establishment and survivorship rates for both Canada and musk thistle. Overall, the soil moisture did not vary significantly between the prairie plots. They all produced similar readings, which can be explained by weather conditions rather than differences in plant diversity. Shallow soil moisture is important during germination and establishment for both thistles, however, by the second growing season the thistles would have established tap roots. I did not have the necessary equipment in the field to take deeper soil moisture readings to determine if plant diversity had an effect on it. As there were no surviving musk thistle or Canada thistle in the high diversity plots at the end of the second season, this suggests the available soil nitrogen is the determining factor in allowing a grassland to resist an invasive species (hypothesis 5).

As the plant diversity increased, the total live biomass increased, however, the low diversity had the highest amount of grass biomass followed by monoculture plots, then the high diversity (hypothesis 3). The light penetration was higher in the high diversity plots, suggesting it had more open spaces compared to the other prairie plots. However, the high diversity plots had the highest amount of live biomass, so the forbs present in these plots must be accounting for the weight difference. The low diversity and monoculture plots had more stands of grasses and higher percent grass cover, which, in the early establishment, was shown to deter thistle establish. Since the low diversity and monoculture plots had surviving thistles and the high diversity plots did not, the percent grass cover effect on thistle resistance decreased by the second growing season.

Finally, as the plant diversity increased, the establishment and survivorship rates for musk thistle and Canada thistle decreased (hypothesis 4). After two growing seasons, in the bare ground control plots, the musk thistles had completed their lifecycles and the Canada thistles were well established. All of the grassland diversity plots had restricted the establishment and reduced the populations of both thistles by the second growing season. The high diversity plots had the lowest establishment rate, for both Canada thistle and musk thistle. The low diversity plots had the second lowest establishment rate followed by the monoculture plots for both thistles types. At the end of the second growing season, musk thistle only persisted in the low diversity and monoculture prairie stands, indicating a higher resistance to thistle survival in the high diversity plots. The Canada thistle only persisted in the monoculture prairie stands, indicating a higher resistance to thistle survival in the high and low diversity plots.

There are a few limitations to this study that need to be addressed. The study only looked at one point during perennial grasslands establishment. It can only infer what would occur if an invasive species is introduced in a grassland just planted or one that is older than four years. This site did not have a true monoculture plot. Although the monoculture plots were only planted with one species, over the past four years, 33 other species emerged and established. Many of these species were actually weeds and native annuals. Based on the results of this experiment, it is probable a true monoculture plot would have more available resources and would have more established thistles. The experiment started just as the prairie research plots had been burned. As a result, all of the diversity plots began with a higher resources availability, which may have affected the establishment rates. At the end of the two growing seasons, all but one of the musk

thistles plants in the prairie plots were still in rosette form. A few Canada thistle bolted in the monocultures, but the majority stayed in rosette form. None of the Canada thistles in the prairie plots began to spread via rhizomes. If the experiment ran longer, it is possible they all would have bolted and completed their reproductive cycle during the following year or had a high over-winter mortality rate, leaving only a few surviving plants. This would have provided interesting data as to the success of the thistles and, possibly, if nutrient limitation was preventing them from reproducing.

Conclusion

Management Implications

It is important to manage for high diversity grasslands, as they are the most resistant to invasive species. It is important to allow for each species to maintain their populations by being able to reproduce each year. Management plans should be tailored for each individual area, be flexible, and employ a variety of techniques, such as patch burn grazing, rotational, and selective grazing, to encourage diversity. Managers need to prevent overgrazing of rangelands and pastures, as this can create a disturbance making resources available for invasives. During years with drought conditions or extremely wet years, prairies are vulnerable to invasives. Areas recovering from a disturbance should be monitored closely for emerging invasive species. If an established prairie is a similar to a monoculture or is low in diversity, additional plantings should be considered for species or functional groups that are minimally present. Not only a variety of grasses but a variety of forbs are important in preventing the establishment of invasive species, as can be seen with our high diversity plots. Having multiple plant species from each functional group leaves less available niches for invasive species, as resources are efficiently utilized.

If musk thistle and Canada thistle are already established in a prairie, then a combination management plan has been shown to be the most effective for control. Monitoring grasslands and rangelands is the first step in prevention and control invasive species. Bare ground and recently disturbed areas should be a priority for monitoring, as my research shows these are the areas where thistles are most likely to establish and become successful. While high diversity areas should be the least vulnerable to invasive

species, this does not mean they should not be monitored, as they can still be susceptible. As it is not always an option for a land manager to routinely check their prairies for new thistle rosettes, and thistles can go unnoticed until they bolt, especially in denser prairies. Once they bolt, they need to be dealt with immediately before the plant reproduces allowing it to establish further. Control methods can vary, but the most successful ones for thistles involve a combination of herbicides and removal. The time of year, the plant's growing stage, surrounding species, and size of the area all need to be taken into consideration when choosing an herbicide and the application method. Musk thistles only need to be removed from the location if there are flower heads on the plants, as the seeds are typically unaffected by the herbicide treatments and can cause new establishments the following year. Canada thistle should be removed or mowed after an herbicide application to prevent further establishment. However, both thistles might require multiple years of management before they are eradicated. If musk thistle and Canada thistle are controlled early in their establishment, less time and money will be wasted managing them.

Future Studies

Future studies with a longer timeframe can assess the success of musk thistle and Canada thistle in a variety of grassland diversity plots. This will allow a researcher to assess if the rosettes still alive in the second growing season are able to reproduce the following year or if nutrients levels prevent it from doing so. Also, it could strengthen the results that nitrogen is the limiting factor for thistle success or indicate if another resources is the cause after two growing seasons. A long term study could see if the seeds and rhizomes planted into the seed bank would emerge in later years due to a

disturbance or fluctuation in nutrients, or if they were not viable when they were planted. More studies need to be completed evaluating the invasibility of grasslands and thistles when they are planted at the same time to evaluate competition for resources. If thistles are able to establish at the same time as native grassland species and they are outcompeting for resources, this can have a huge effect on the success of the grassland or rangeland. These studies can be used to determine the amount of time and how intensely a grassland needs to be managed once it is planted before it is able to resist invasives on its own. It is important to do a study similar to this one at a site that has not just been burned when the thistles are planted. It is possible the establishment and survivorship rates would have been lower if the thistles were planted in a fully grown prairie.

Conclusion

Two pieces of evidence support nitrogen availability as the as the key resource that allows for grasslands to resist invasive species during establishment and later subsequent survival. The step wise regression showed during the establishment phase the available nitrogen was the best predictor of thistle success. The ANOVA tested for available nitrogen over two growing seasons showed significance by treatment type, however, only the bare ground was significantly different from the other treatments. Overall it seems the less available soil nitrogen, the less likely a musk thistle or Canada thistle will establish and survive. Further research into the effects of nitrogen availability could lead to a better understanding of grassland resistance to invasive species, as well as, determining a threshold for the most resistance. The bare ground control plots, which had the most available resources, also had the most successful thistles overall. This shows that recently disturbed areas, or newly open bare ground, have the highest risk for a musk

thistle or Canada thistle invasion. It is important to manage for a grassland community with high diversity as there are less available resources for an introduced species. By managing for highly diverse grasslands, which are more resistant to invasives, land managers save time and money that would have to go into controlling an invasive species. Invasive species have been found in high diversity grasslands and some low diversity grasslands have no invasives, however, high diversity grasslands have a lower chance of invasibility than lower diversity levels.

Literature Cited

Booth, Barbara D., Stephen D. Murphy, and Clarence J. Swanton. 2010. *Invasive Plant Ecology in Natural and Agricultural Systems*. CABI.

Burke M.J.W., and J.P Grime. 1996. An experimental study of plant community invasibility. *Ecology* 77: 776–790.

Davis M.A. and M. Pelsor. 2001. Experimental support for a resource-based mechanistic model of invasibility. *Ecology Letters* 4: 421–428.

Davis M.A., P. Grime, and K. Thompson. 2000. Fluctuating resource in plant communities: a general theory of invasibility. *Journal of Ecology* 88:528-534.

Funk, Jennifer L. and Peter M. Vitousek. 2007. Resource-use efficiency and plant invasion in low resource systems. *Nature* Vol. 446 April 2007.

Gross, Katherine L., Gary G. Mittelbach. And Heather L. Reynolds. 2005. Grassland Invasibility and Diversity: Responses to Nutrients, Seed Input, and Disturbance. *Ecology*. 86(2): 476-486.

Holzmueller Eric J, and Shibu Jose. 2009. Invasive plant conundrum: What makes the aliens so successful? *Journal of Tropical Agriculture* 47 (1-2): 18-29.

Huenneke L.F., S.P. Hamburg. R. Koide, H.A. Mooney, and P.M Vitousek.1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology* 71: 478–491.

NISIC. 2012. What is an Invasive Species? *National Invasive Species Information Center***.** http://www.invasivespeciesinfo.gov/whatis.shtml.

NOAA. 2015. Wood River, Nebraska. *National Oceanic and Atmospheric Administration's National Climatic Data Center.* www.ncdc.noaa.gov.

Oelmann, Yvonne, Wolfgang Wilcke, Vicky M. Temperton, Nina Buchmann, Christiane Roscher, Jens Schumacher, Ernst-Detlef Schulze and Wolfgang W. Weisser. 2007. Soil and Plant Nitrogen Pools Related to Plant Diversity in an Experimental Grassland. *Soil Science Society of America Journal* 71 (3):720-729.

Parendes L.A. and J.A. Jones. 2000. Role of light availability and dispersal in exotic plant invasion along roads and streams in the H. J. Andrews Experimental Forest, Oregon. *Conservation Biology* 14: 64–75.

Pimm, Stuart L. 1989. *Theories of Predicting Success and Impact of Introduced Species Biological Invasions: a Global Perspective.* John Wiley & Sons Ltd.

Roeth, Fred, Steven R. Melvin and Irvin L. Schleufer. 2003. Noxious Weeds of Nebraska: Musk Thistle. *University of Nebraska- Lincoln Digital Commons*.

Simberloff, Daniel. 2013. *Invasive Species: What Everyone Needs to Know*. Oxford University Press. New York, New York.

Simberloff, Daniel, Lara Souza, Martin A. Nunez, M. Noelia Barrios-Garcia and Windy Bunn. 2012. The natives are restless, but not often and mostly when disturbed. *Ecology*. 93(3) 598-607.

Tilman, David. 1997. Community invisibility, recruitment limitation and grassland biodiversity. *Ecology* 78(1) pp.81-92.

Tilman, D, .J. Knops, D. Wedin and P. Reich. 2002. Plant diversity and composition: effects on productivity and nutrient dynamics of experimental grasslands. Pages 21-35 in M. Loreau, S. Naeem, and P. Inchausti (eds.). *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives.* Oxford University Press.

Tilman, D., D. Wedin and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*. 379:718-720.

Theoharides, Kathleen A. and Jeffery S. Dukes. 2007. Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist*. 176:256-273.

Web Soil Survey. 2015. Wood River, Nebraska. *National Cooperative Soil Survey. Natural Resources Conservation Service.* http://websoilsurvey.nrcs.usda.gov

Figures

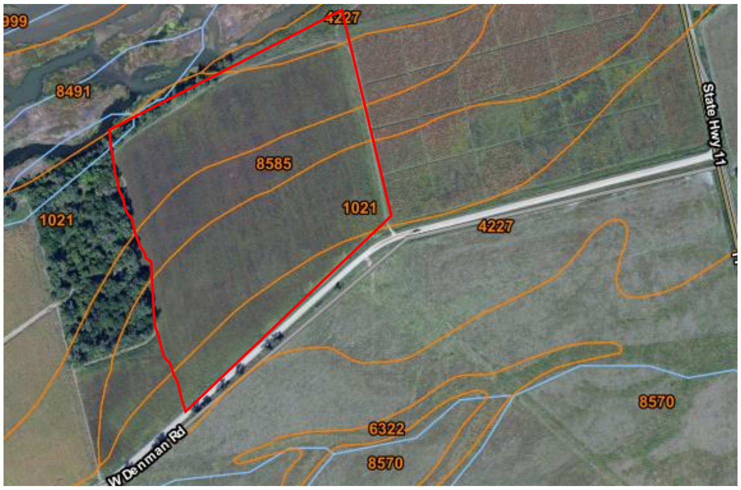


Figure 3: Soils map of The Nature Conservancy, Wood River, research plots (red polygon) from Natural Resource Conservation Service.

Map Unit Symbol	**Soil Description**
1021	Caruso loam, rarely flooded
8585	Wann loam, rarely flooded
4227	Bolent-Calamus complex, occasional flooded

Figure 4: Aerial view of The Nature Conservancy's research plots planted in 2010 with diversity treatments labeled as the follows: HD: High Diversity, LD: Low Diversity, Mono: Monoculture Plots.

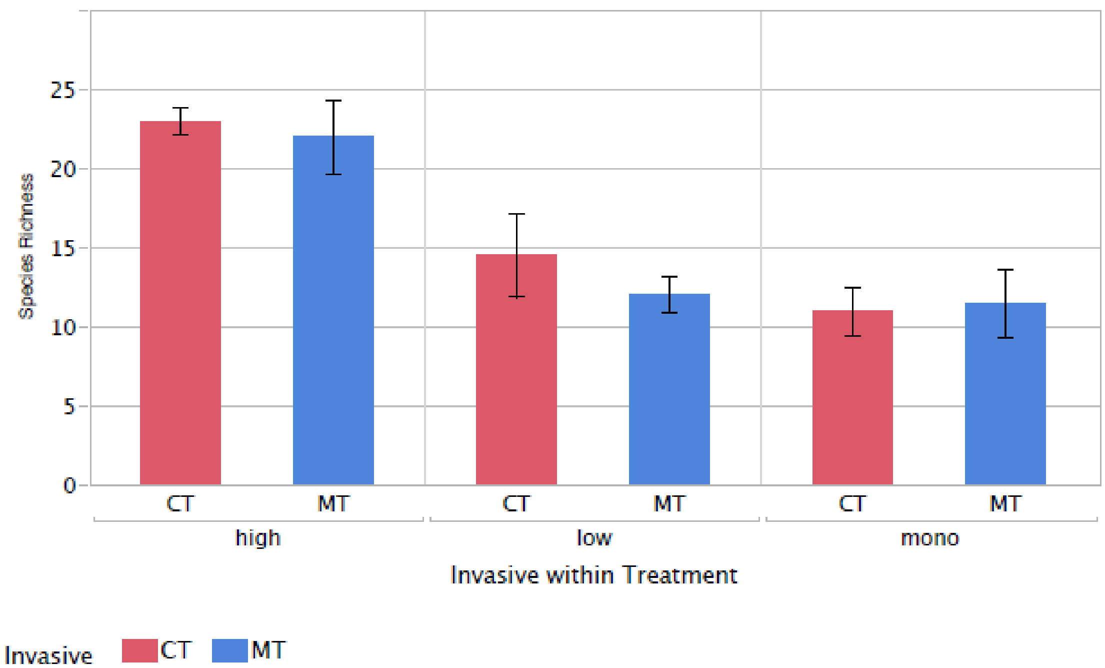


Figure 5A: The average number of species found in the thistle subplot (CT- Canada thistle, MT- Musk thistle) within diversity treatments (July 2013) (Std. err. 1).

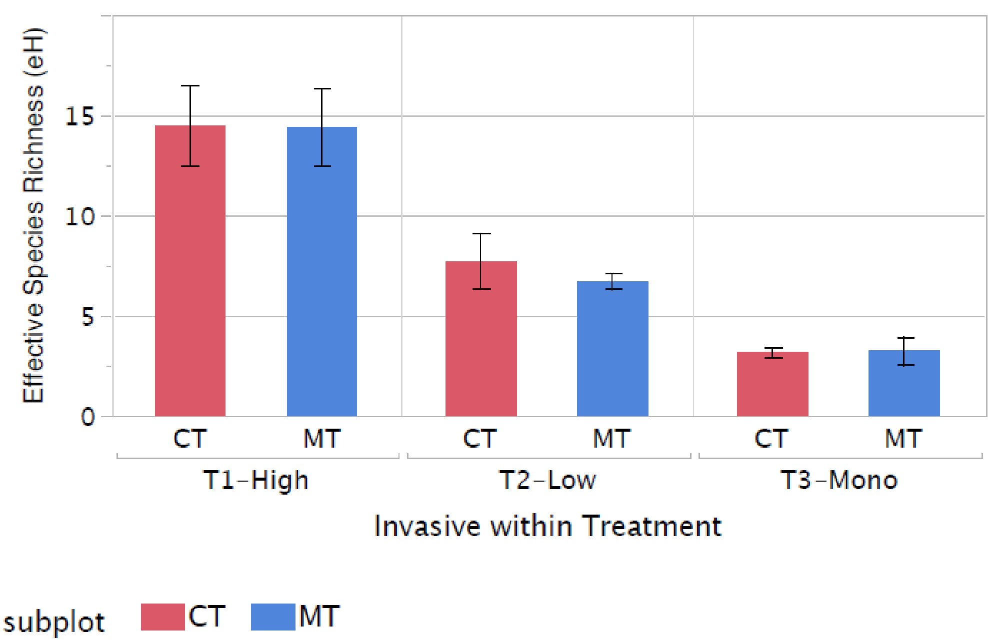


Figure 5B: The average effective species richness (e^H) found in the thistle subplot (CT- Canada thistle, MT- Musk thistle) within diversity treatments (July 2013) (Std. err. 1).

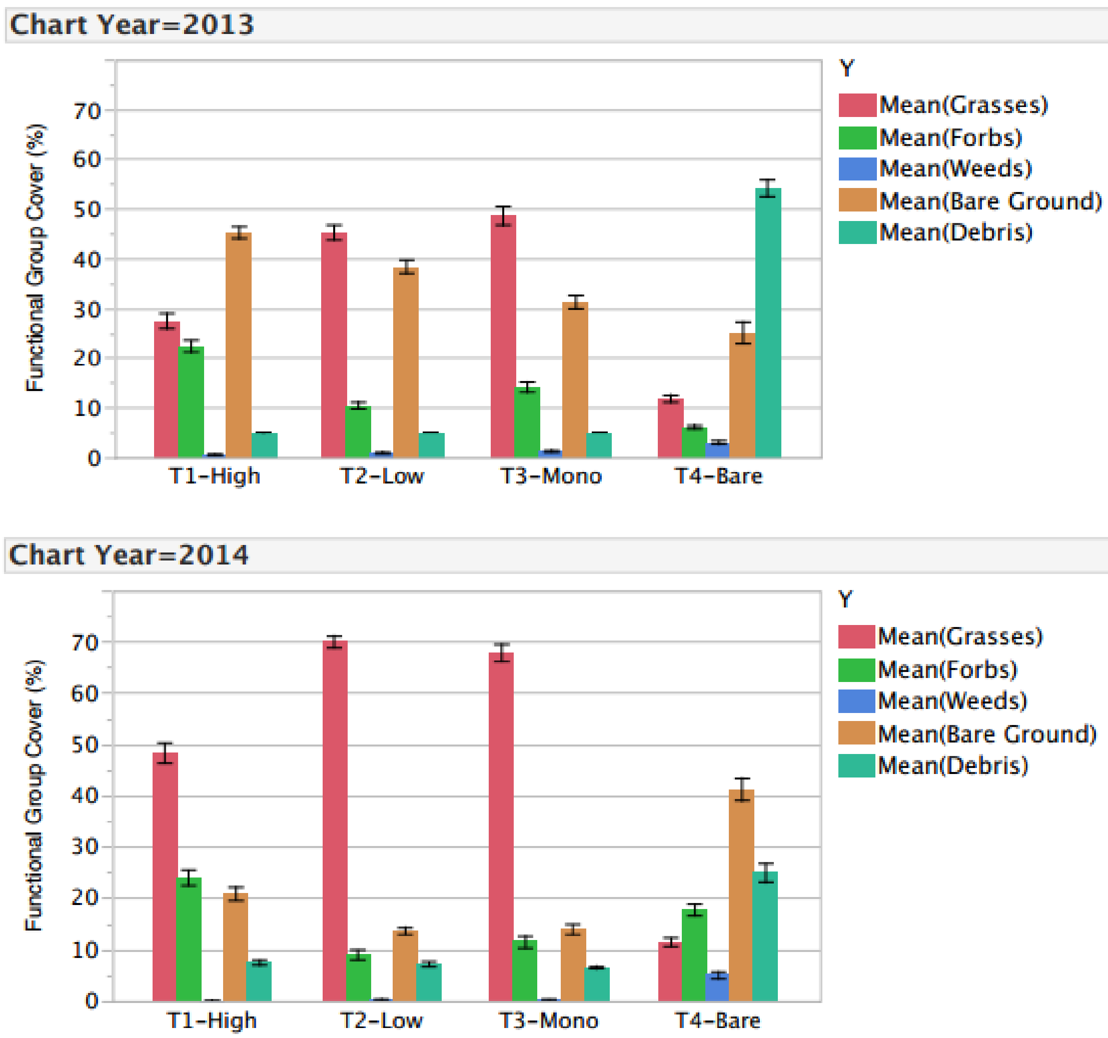


Figure 6: The average plant composition by functional group between mid-June thru mid-August for 2013 and 2014. (Std. err. 1). Weeds in the legend refers to the two experimental thistle species.

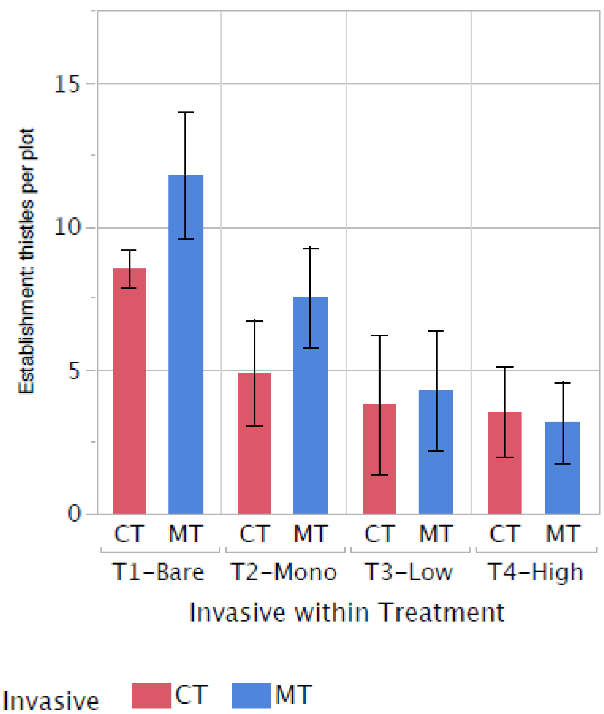


Figure 7A: The average number of established seedlings for musk thistle (MT) and Canada thistle (CT) in July 2013 (3 months after planting) for each diversity treatment. (Std. err. 1)

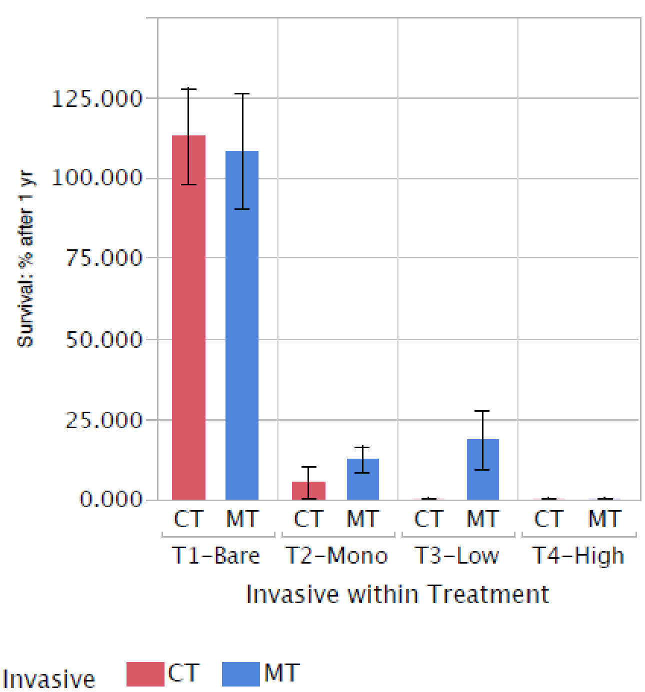


Figure 7B: The average number of musk and Canada thistles in September 2014 relative to July 2013 abundance (17 months after planting) for each diversity treatment (means and Std. err. 1).

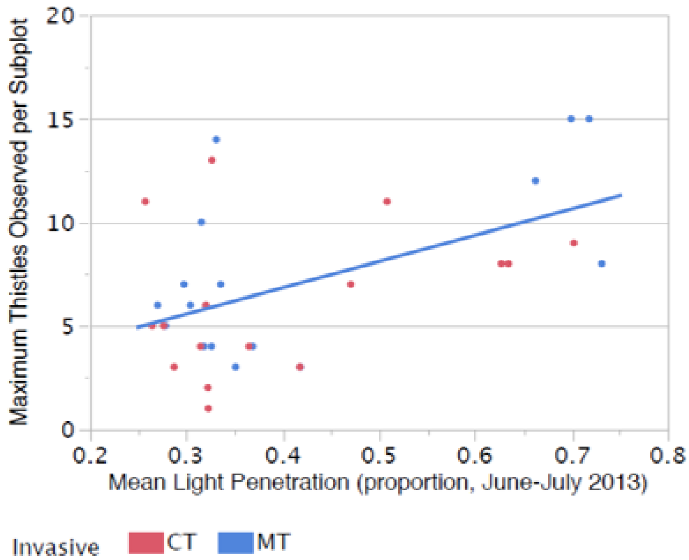


Figure 8: Average Light Penetration: Seedling abundance versus average light penetration beneath the vegetation (June – July 2013) for musk thistle (MT) and Canada thistle (CT) subplots. R^2 =0.261, P-value =0.0028. Treatments were not statistically different from each other, only one fit line was added.

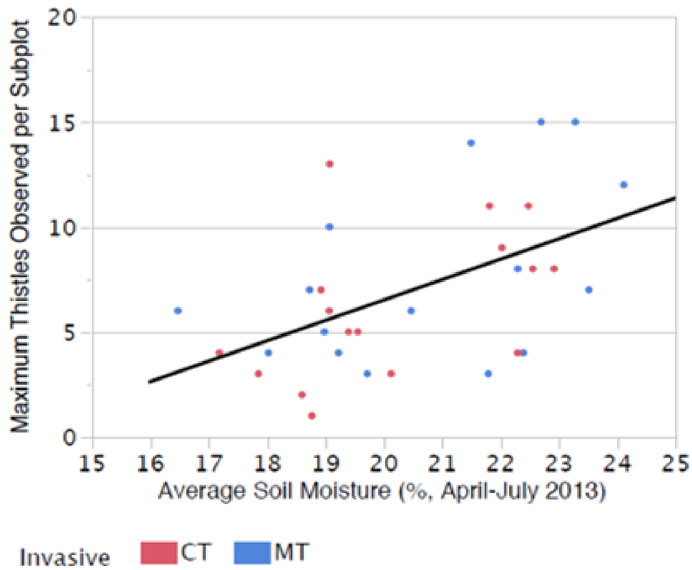


Figure 9: Average Soil Moisture: Seedling abundance versus average soil moisture to 5 cm depth during seedling establishment (April – July 2013) for musk thistle (MT) and Canada thistle (CT) subplots. R^2 =0.262, P-value =0.003. Treatments were not statistically different from each other, only one fit line was added.

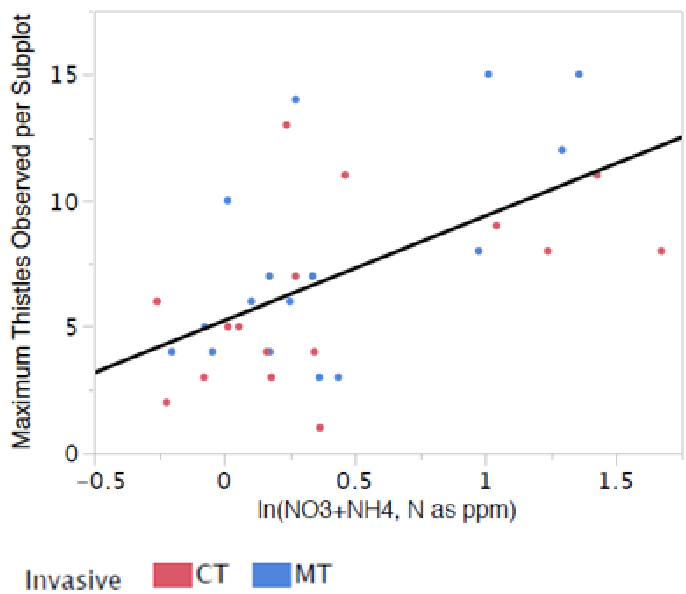


Figure 10: Seedling abundance versus soil N availability (June – July 2013) for musk and Canada thistle. Curves are based on linear fits of thistle number versus Log-transformed $NO_3^{-} + NH_4^{+}$. R^2 =0.323, P-value =0.0007. Treatments were not statistically different from each other, only one fit line was added.

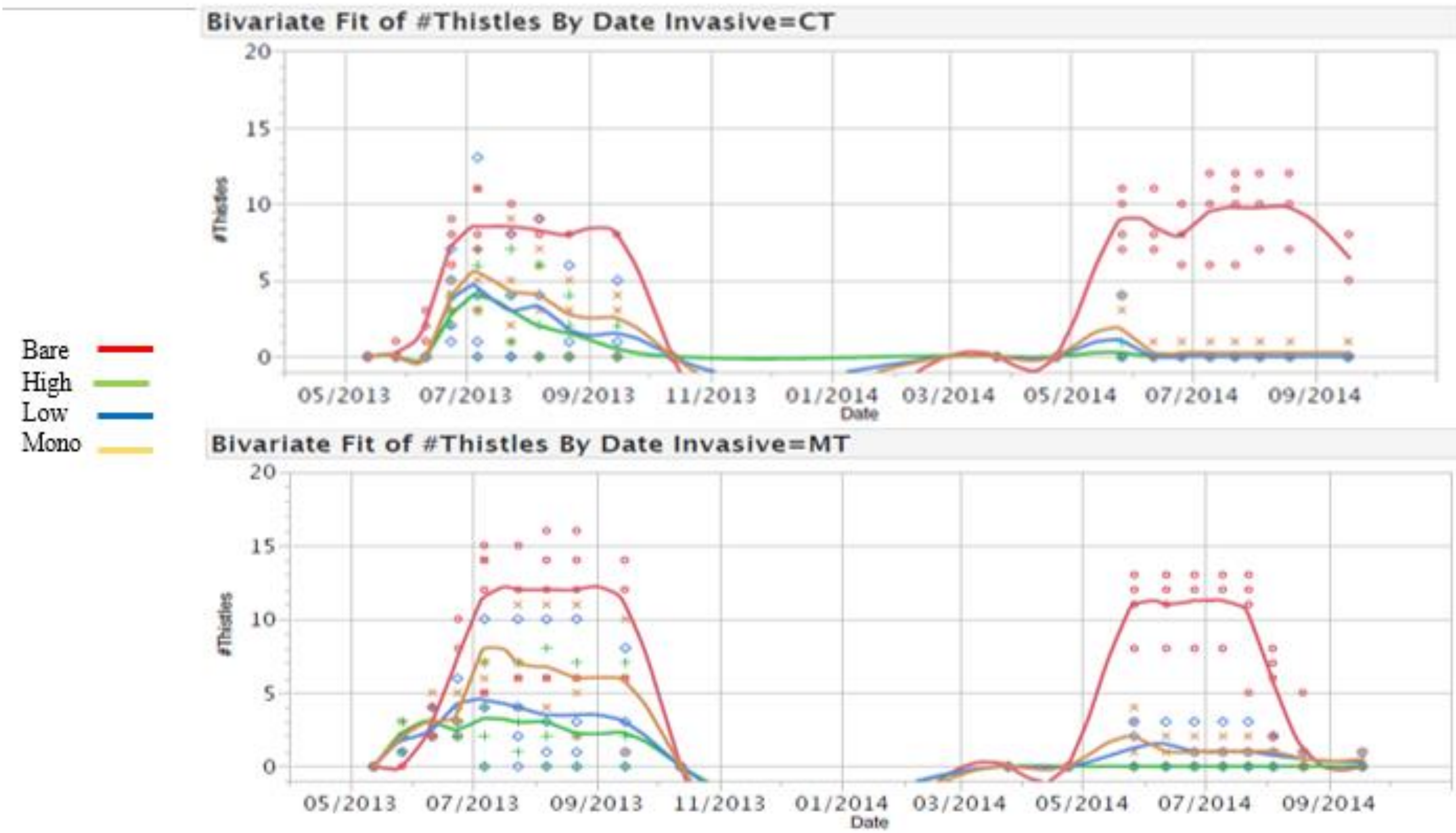


Figure 11: The total number of Canada thistle (CT) and musk thistle (MT) per diversity type over two years (2013, 2014). The lines display the running average for each diversity type on a given day.

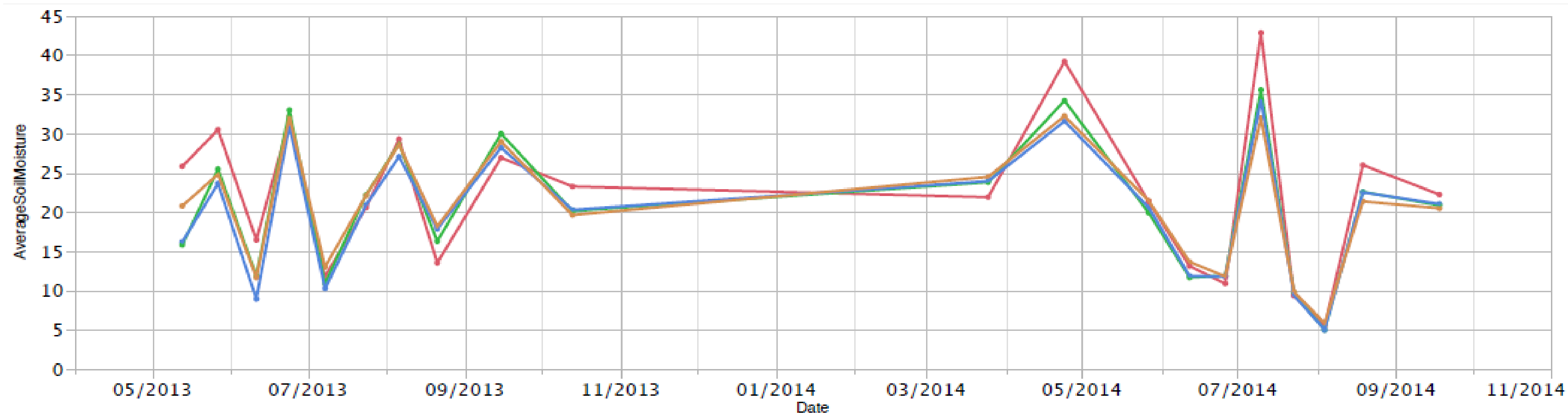


Figure 12: The average soil moisture, over two growing seasons (2013, 2014) for each diversity type.

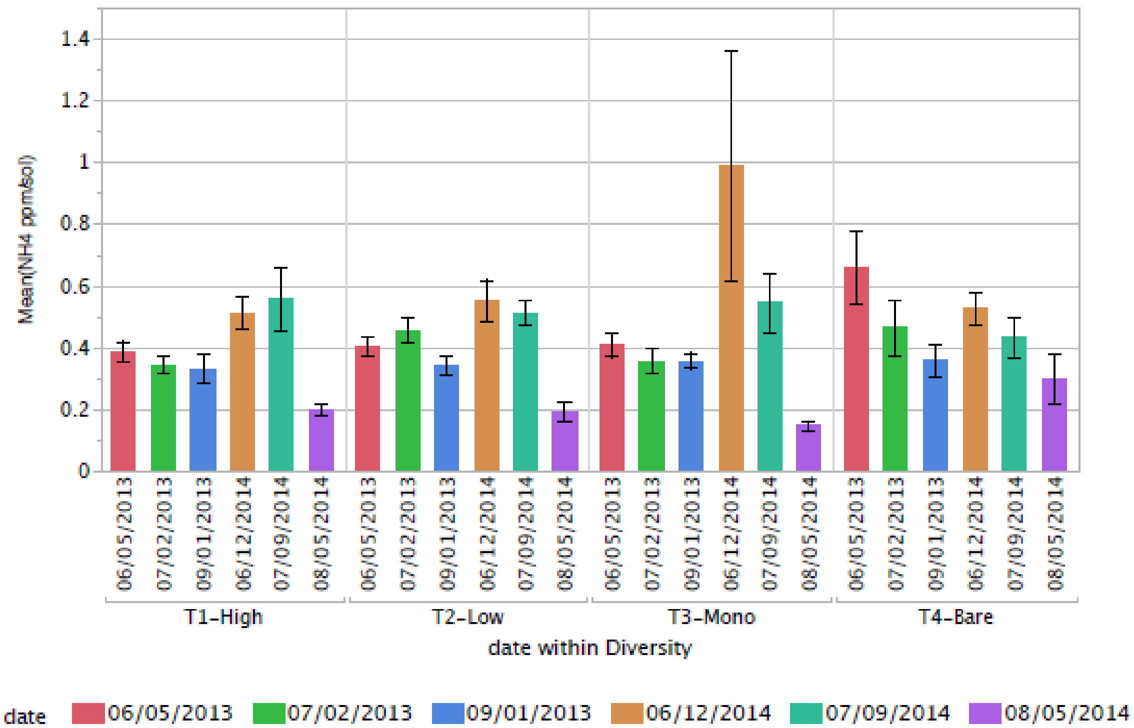


Figure 13: The average available NH_4^+ (ppm) in the soil, over two growing seasons (2013, 2014), for each diversity type. (Std. err. 1)

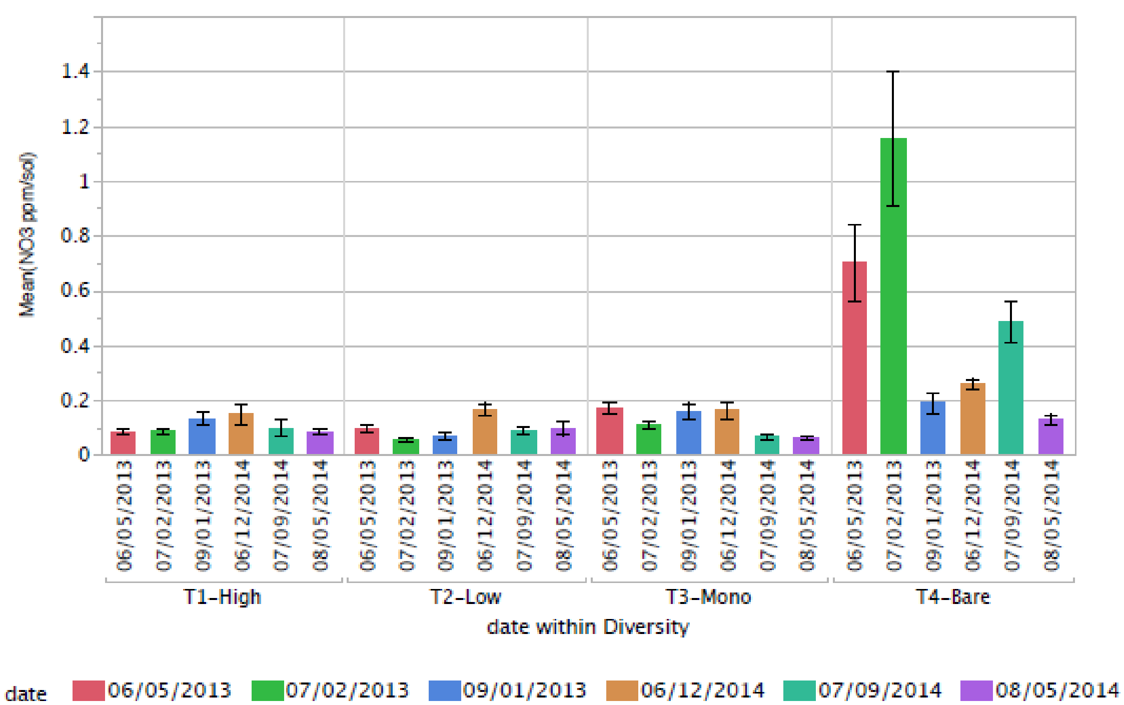


Figure 14: The average available NO_3^{--} (ppm) in the soil, over two growing seasons (2013, 2014), for each diversity type. (Std. err. 1)

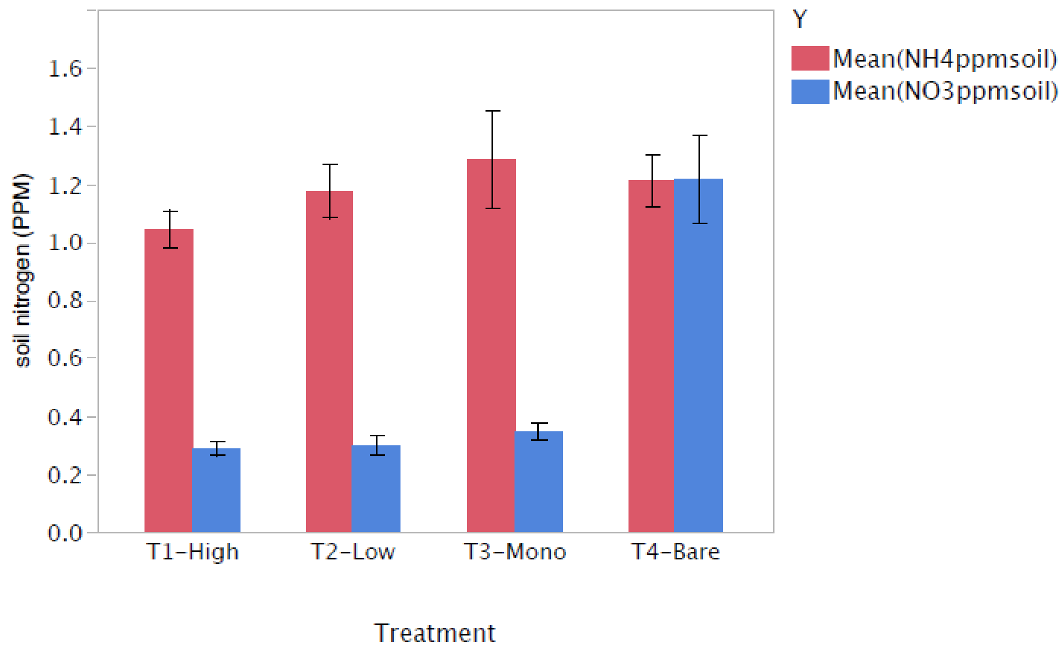


Figure 15: Average available soil nitrogen (ppm) for NH_4^+ and NO_3^{--}, over the two growing seasons (2013, 2014) by diversity type. (Std. err. 1)

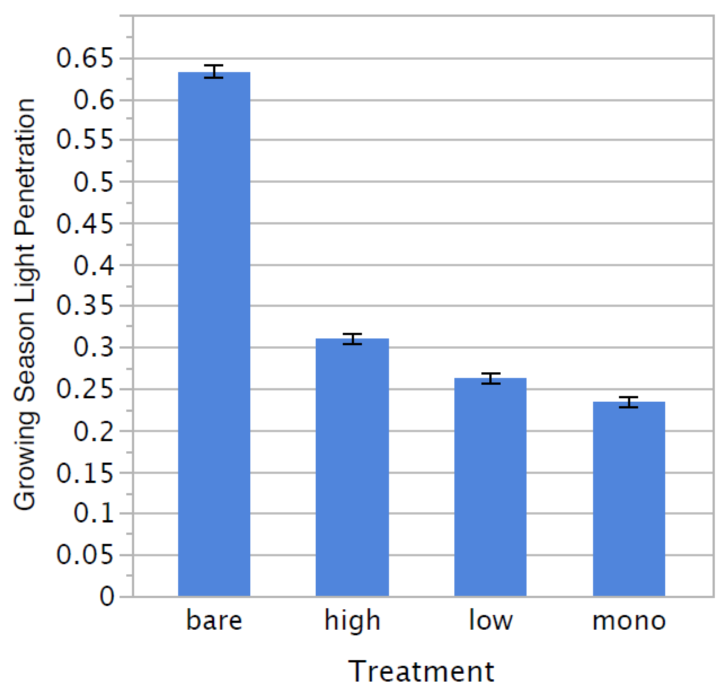
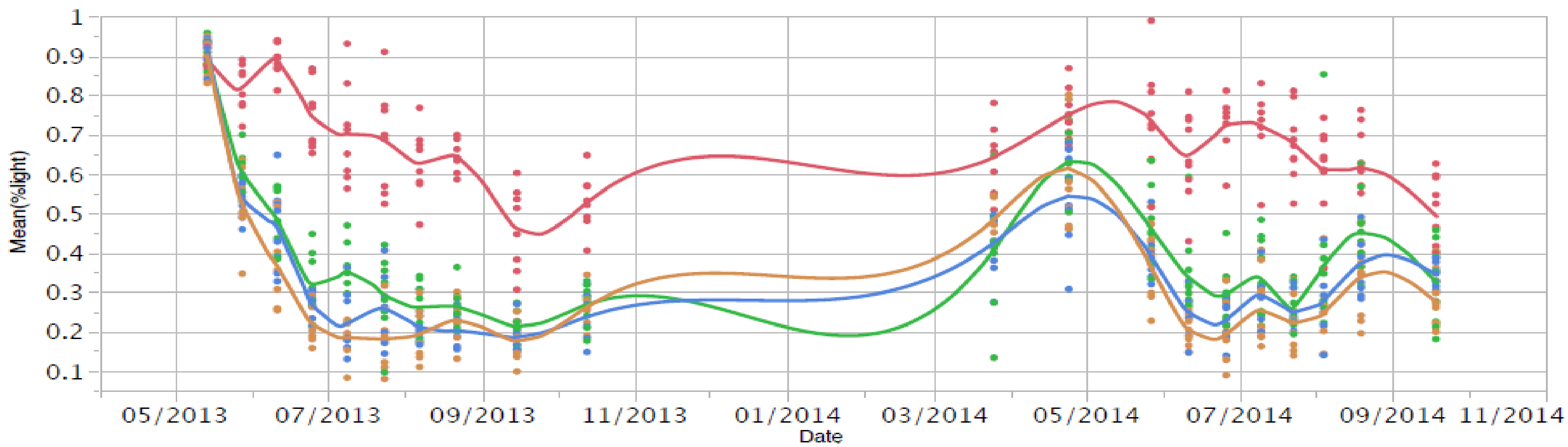


Figure 16: Average light penetration (%) by diversity type over both growing seasons, mid-June to September (2013, 2014) (Std. err. 1)



Bare
High
Low
Mono

Figure 17: Average percent light (%) for each treatment by date for both growing seasons (2013, 2014). The lines represent the running average for each diversity type.

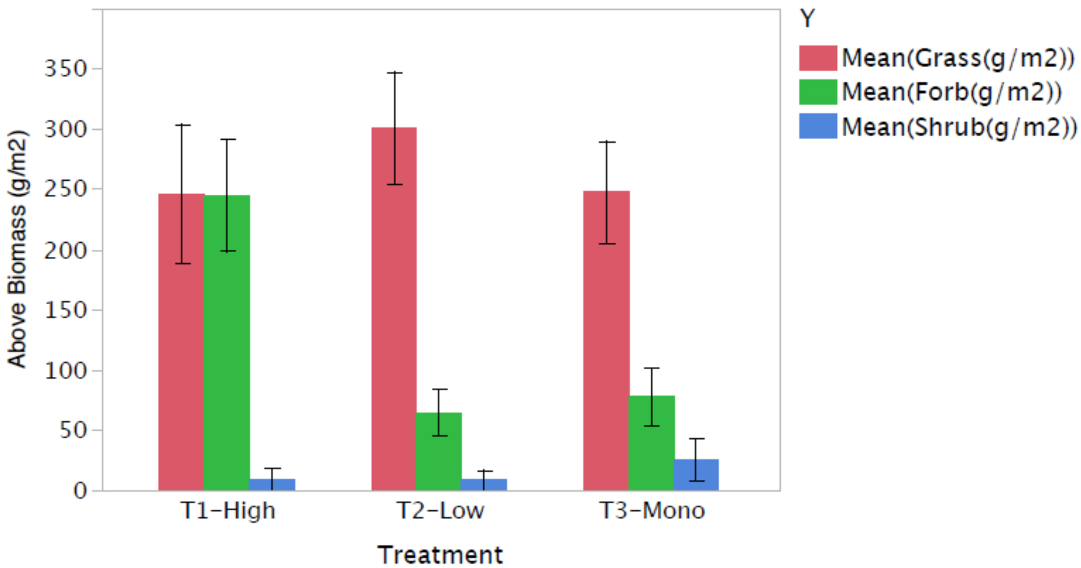


Figure 18: Average aboveground biomass (g/m^2) for each diversity treatment by plant composition group, August 2014. (Std. err. 1)

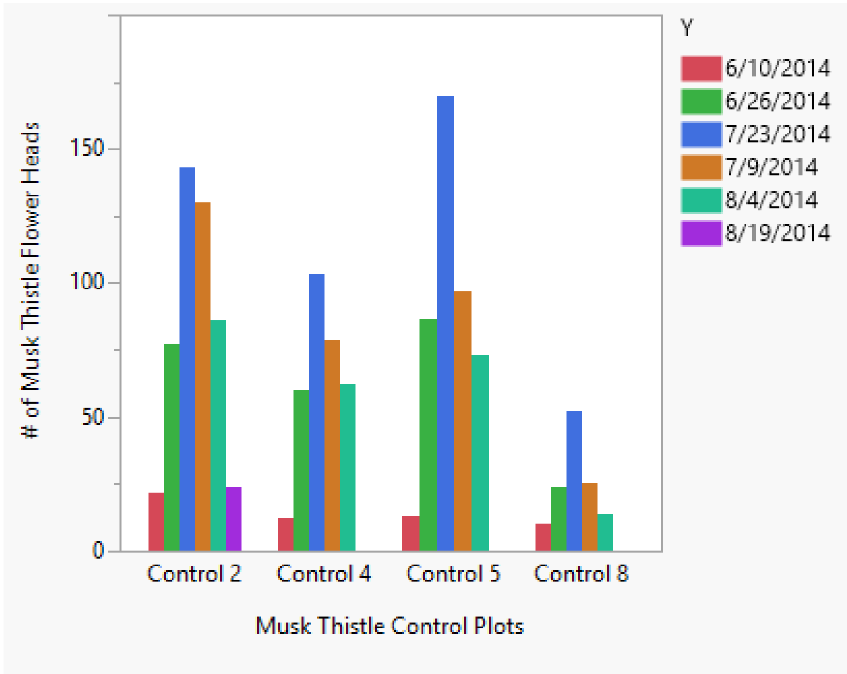


Figure 19: The number of musk thistles flower heads for the four bare ground control plots by date.

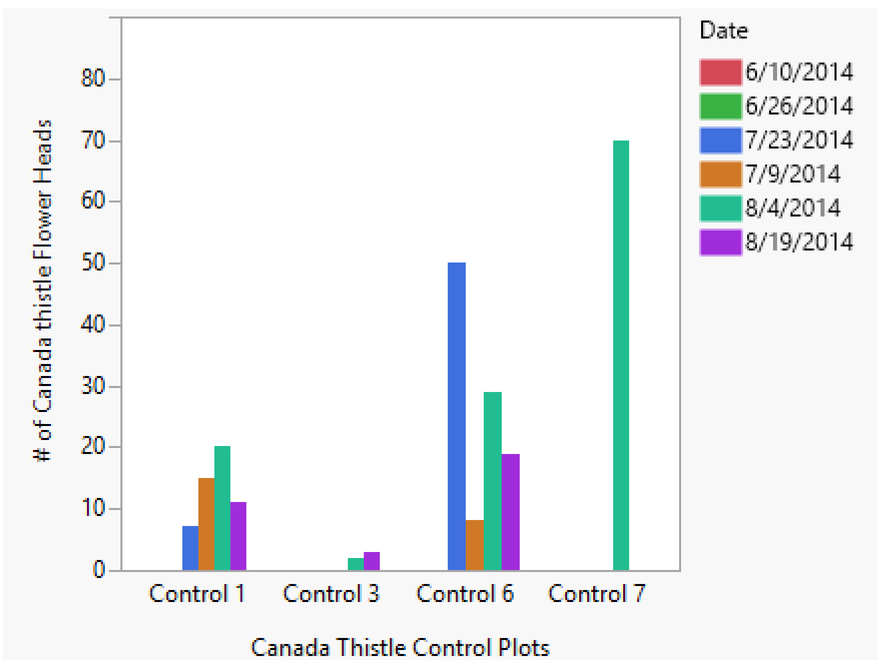


Figure 20: The number of Canada thistles flower heads for the four bare ground control plots by date.

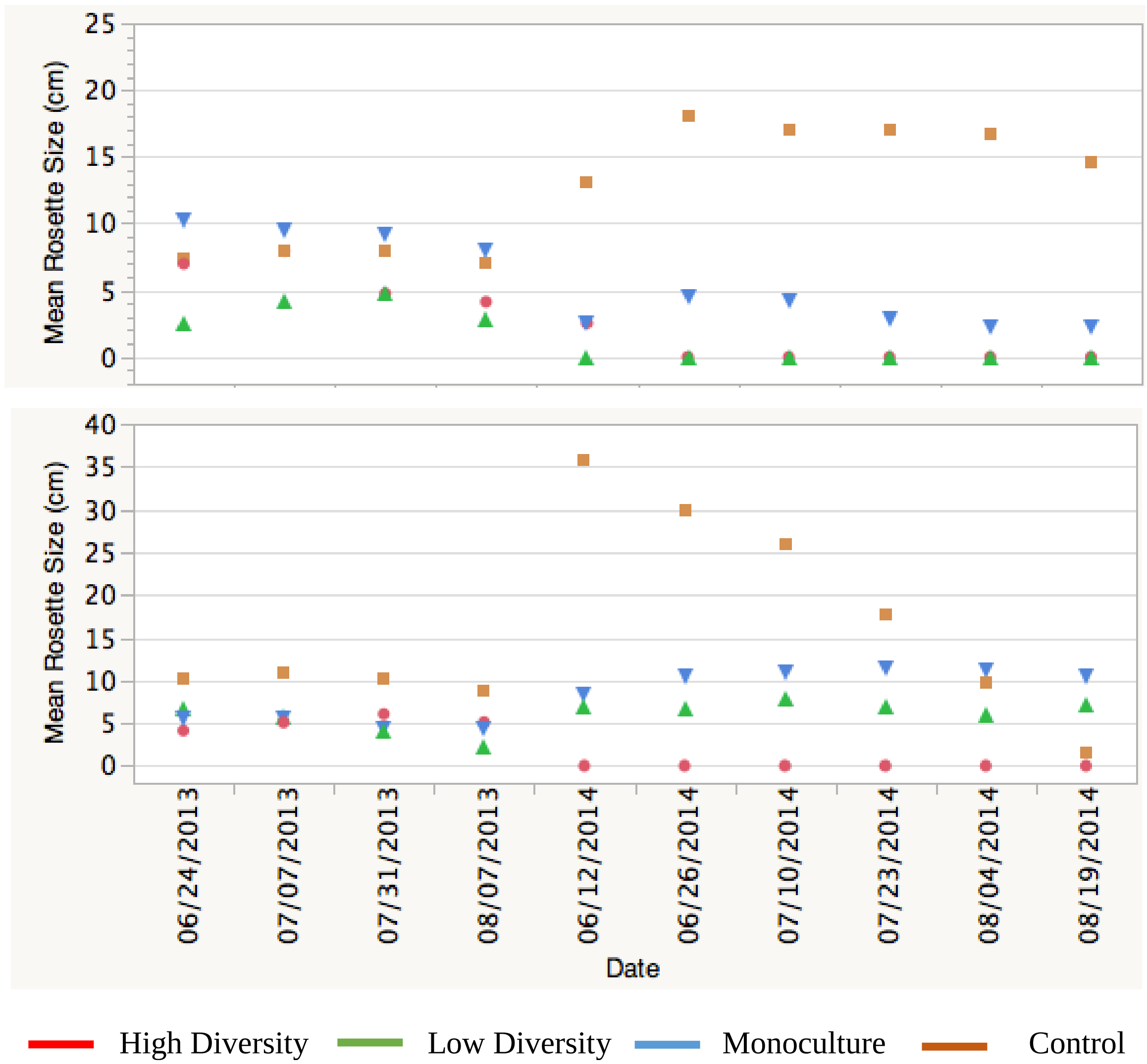


Figure 21: The top graph represents Canada thistle average rosette size (cm) for each date by diversity type. The bottom graph represents Musk thistle average rosette size (cm) for each date by diversity type.

Tables

Treatment	Species	Mean Cover Class
T1-Mono	*Andropogon gerardii*	4.25
T1-Mono	*Chenopodium album*	0.8125
T1-Mono	*Solidago missouriensis*	0.8125
T1-Mono	*Taraxacum officinale*	0.625
T1-Mono	*Solidago canadensis*	0.59375
T1-Mono	*Setaria sp.*	0.40625
T1-Mono	*Cirsium arvense*	0.25
T1-Mono	*Solanaceae species*	0.1875
T1-Mono	*Carduus nutans*	0.15625
T1-Mono	*Lacutca serr.iola*	0.15625
T1-Mono	*Abutilon theophrasti*	0.125
T1-Mono	*Ambrosia psilostachya*	0.125
T1-Mono	*Cirsium vulgare*	0.125
T1-Mono	*Conyza canadensis*	0.125
T1-Mono	*Physalis virginicus*	0.125
T1-Mono	*Cirsium altissimum*	0.09375
T1-Mono	*Gaura sp.*	0.09375
T1-Mono	*Rumex altissimus*	0.09375
T1-Mono	*Bromus inermis*	0.0625
T1-Mono	*Chenopodium sp.*	0.0625
T1-Mono	*Erigeron annuuss*	0.0625
T1-Mono	*Poa pratensis*	0.0625
T1-Mono	*Verbena stricta*	0.0625
T1-Mono	*Conium maculatum*	0.03125
T1-Mono	*Erigeron philadelphicus*	0.03125
T1-Mono	*Hackelia virginiana*	0.03125
T1-Mono	*Hordeum jubatum*	0.03125
T1-Mono	*Lotus unifoliolatus*	0.03125
T1-Mono	*Lythrum salicaria*	0.03125
T1-Mono	*Medicago lupulina*	0.03125
T1-Mono	*Setaria viridis*	0.03125
T1-Mono	*Vernonia fasciculata*	0.03125
T1-Mono	*Celtis sp.*	0.03125
T1-Mono	*unidentified vine*	0.03125
T2-Low	*Sorghastrum nutans*	2.15625

Cover Class Legend	
Cover Class	**Percent Cover Midpoint Range**
1	2.5
2	15
3	37.5
4	62.5
5	85
6	97.5

T2-Low	*Elymus canadensis*	1.9375
T2-Low	*Andropogon gerardii*	1.78125
T2-Low	*Chenopodium album*	0.96875
T2-Low	*Panicum virgatum*	0.8125
T2-Low	*Taraxacum officinale*	0.375
T2-Low	*Solidago missouriensis*	0.28125
T2-Low	*Cirsium altissimum*	0.25
T2-Low	*Gaura sp.*	0.25
T2-Low	*Cirsium arvense*	0.21875
T2-Low	*Schizachrium scoparium*	0.21875
T2-Low	*Setaria sp.*	0.21875
T2-Low	*Solidago canadensis*	0.21875
T2-Low	*Helianthus annuus*	0.1875
T2-Low	*Aster lac.*	0.15625
T2-Low	*Conyza canadensis*	0.125
T2-Low	*Verbena stricta*	0.125
T2-Low	*Elymus trachycaulus*	0.09375
T2-Low	*Helianthus maximilliani*	0.09375
T2-Low	*Helianthus pauciflorus*	0.09375
T2-Low	*Monarda fistulosa*	0.09375
T2-Low	*Physalis virginicus*	0.09375
T2-Low	*Ambrosia psilostachya*	0.0625
T2-Low	*Eupatorium altissimum*	0.0625
T2-Low	*Lacutca serr.iola*	0.0625
T2-Low	*Penstemon digitalis*	0.0625
T2-Low	*Rudbeckia hirta*	0.0625
T2-Low	*Amorpha canescens*	0.03125
T2-Low	*Chenopodium sp.*	0.03125
T2-Low	*Conium maculatum*	0.03125
T2-Low	*Cornus Species*	0.03125
T2-Low	*Digitaria cognata*	0.03125
T2-Low	*Eragrostis trichodes*	0.03125
T2-Low	*Erigeron sp.*	0.03125
T2-Low	*Lactuca ludoviciana*	0.03125
T2-Low	*Lotus unifoliolatus*	0.03125
T2-Low	*Muhlenbergia racemosa*	0.03125
T2-Low	*Oenothera sp.*	0.03125
T2-Low	*Oxalis stricta*	0.03125
T2-Low	*Polygonum scandens*	0.03125
T2-Low	*Tagopogon dubius*	0.03125
T3-High	*Elymus canadensis*	1.75

T3-High	*Sorghastrum nutans*	1.34375
T3-High	*Andropogon gerardii*	1.28125
T3-High	*Panicum virgatum*	1
T3-High	*Muhlenbergia racemosa*	0.90625
T3-High	*Ratibida columnifera*	0.90625
T3-High	*Chenopodium album*	0.8125
T3-High	*Solidago canadensis*	0.8125
T3-High	*Achillea millefolium*	0.71875
T3-High	*Helianthus maximilliani*	0.59375
T3-High	*Lotus unifoliolatus*	0.5625
T3-High	*Rudbeckia hirta*	0.5625
T3-High	*Solidago missouriensis*	0.46875
T3-High	*Elymus trachycaulus*	0.40625
T3-High	*Schizachrium scoparium*	0.40625
T3-High	*Setaria sp.*	0.40625
T3-High	*Monarda fistulosa*	0.34375
T3-High	*Sporobolus compositus*	0.34375
T3-High	*Eragrostis trichodes*	0.28125
T3-High	*Silphium integrifolium*	0.28125
T3-High	*Koeleria pyramidata*	0.25
T3-High	*Gaura sp.*	0.21875
T3-High	*Taraxacum officinale*	0.1875
T3-High	*Ambrosia trifida*	0.15625
T3-High	*Conyza canadensis*	0.15625
T3-High	*Solidago rigida*	0.15625
T3-High	*Bromus japonicus*	0.125
T3-High	*Carex brevior*	0.125
T3-High	*Chenopodium sp.*	0.125
T3-High	*Cirsium altissimum*	0.125
T3-High	*Oenothera sp.*	0.125
T3-High	*Penstemon grandiflorus*	0.125
T3-High	*Solanaceae species*	0.125
T3-High	*Bromus tectorum*	0.09375
T3-High	*Lactuca ludoviciana*	0.09375
T3-High	*Aster lac.*	0.0625
T3-High	*Astragalus canadensis*	0.0625
T3-High	*Cirsium arvense*	0.0625
T3-High	*Cornus Species*	0.0625
T3-High	*Digitaria cognata*	0.0625
T3-High	*Helianthus pauciflorus*	0.0625
T3-High	*Penstemon gracilis*	0.0625

T3-High	*Potentilla arguta*	0.0625
T3-High	*Verbena stricta*	0.0625
T3-High	*Erigeron sp.*	0.0625
T3-High	*Asclepias verticillata*	0.03125
T3-High	*Cirsium vulgare*	0.03125
T3-High	*Dalea candidum*	0.03125
T3-High	*Dalea purpureum*	0.03125
T3-High	*Hackelia virginiana*	0.03125
T3-High	*Helianthus laetiflorus*	0.03125
T3-High	*Melilotus officinalis*	0.03125
T3-High	*Pascopyron smithii*	0.03125
T3-High	*Penstemon digitalis*	0.03125
T3-High	*Schrankia nuttallii*	0.03125

Table 1: The species list shows the specific species found in all of the subplots by diversity type, as well as, the mean cover class based on 24 replicates of Daubenmire quadrats taken within the subplots (July 2013).

	R^2	N	Treatment		Date		Treatment *Date	
			F- Value	DF	F- Value	DF	F- Value	DF
Light Penetration	0.52	2753	955.75***	3	22.12***	11	8.4***	33
Soil Moisture	0.96	384	3.6*	3	719.35***	11	6.07***	33
LnNH_4^++ NO_3^-	0.55	192	22.59***	3	9.12***	5	8.91***	15

Legend: *P< .05, **P<.01, ***P< .0001

Table 2: Individually analyzed for percent light penetration, soil moisture and nitrogen, the two-way ANOVA results tested the effects of treatment, date, and treatment by date interaction values over both growing seasons (2013, 2014) for all diversity types.

Chapter 3:

MUSK THISTLE ESTABLISHMENT RESPONSE TO WATER LIMITATIONS

Katilyn Price

Introduction

Carduus nutans, Musk thistle is a biennial invasive plant with a deep taproot that reproduces through seeds. It is found throughout grasslands, rangelands, roadsides, and recently disturbed areas. It reduces forage and crop production, as well as, degrades grasslands. Based off observations in field studies, musk thistles established and grew better in bare ground plots versus any plots with vegetation present. The bare ground control plots had the most available resources, including moisture, nitrogen, and light. Determining the limiting factor during the early establishment phase can help establish what bare ground sites are the most vulnerable to musk thistle. Light and nitrogen are generally not limiting factors in the early stages of germination and emergence (McCarty et al. 1969, Peterson-Smith and Shea 2010, Ruggiero and Shea 2011). Previous studies have shown soil moisture to be an important factor for musk thistle seedling emergence (Ruggiero and Shea 2011, Doing et al. 1969, Lee and Hamrick 1983, McCarty et al. 1969). Musk thistle seeds require three to five days of moist soil before germination begins (McCarty et al. 1969, Lee and Hamrick 1983, Lee and Hamrick 1987). A terrarium experiment was designed, with previous studies in mind, to test soil moisture as the limiting factoring, providing high nutrient soil, no below ground root competition and plenty of light. The objective of this experiment was to determine the establishment rates and success of musk thistle (Carduus nutans) when water restrictions are in place. This experiment would determine thistle success by the number of establish thistles per water treatment level and measuring the diameter of the thistles. I hypothesized the musk thistle establishment and growth of rosettes would increase as the water increased. I hypothesized there would be less excess water in the treatment receiving the highest

amount of water as the rosettes would be utilizing it. I hypothesized the germination rate would increase as the amount of water increases.

Method and Materials

Experimental Design

The experiment was conducted at the North Platte Western Extension and Research Center in a terrarium (Figures 22 and 23). The terrarium was separated into four large chambers each containing three sections, with the following dimensions L: 55.8 cm H: 31.75 cm W: 24.13 cm, allowing for four replications of three different treatments (Figure 23). An EC-5 Volumetric Soil Moisture Sensor was placed within each of the twelve sections located in the terrarium. The sensors were connected to Em50 Data Logger by Decagon Devices, which were set up to take a soil moisture reading every hour. At the beginning of the experiment, all sections in the terrarium were evenly filled with Schutz Moisture Plus Potting Mixture. The soil surface was 5 cm below the dividers in each section to prevent possible soil moisture contamination. The room was set up to have a 15 hour day using artificial lighting, and maintaining the temperature at 22.2°C. The goal was to have three musk thistle plants growing in each section of the chamber, a total of twelve musk thistle rosettes for each treatment type. In order to achieve this, the sections were divided into thirds and at the center of each sub-section three musk thistle seeds (amount determined from previous germination studies) were planted at a 0.6 cm depth in June 2014. No more than three musk thistles grew in each section, therefore, no plants were removed to prevent additional competition. The chamber doors were left open at all times to prevent the buildup of condensation on the sides of the terrarium, which could have altered the water restrictions in the sections. Twice a week, each section was watered by hand using a premeasured container with either 0.38, 0.76, 1.1 litters (0.1, 0.2, 0.3 gallons respectively) of water, thus creating four replicates of each

treatment. These treatments are referred to as low, medium, and high. Once a week, a single soil moisture reading was collected just after watering. The diameter (cm) of the rosette was measured from the tips of the largest green leaves. At the end of the experiment in October 2014, the soil moisture sensor data loggers were removed and downloaded to a computer for analysis.

Data Analysis

The soil moisture data that was taken hourly, was averaged over each day. Then averaged for each day by treatment type, low, medium, and high before analysis. The means for each treatment for each day were then graphed to examine overall trends. The thistle rosette sizes were graphed by treatment to examine for trends, as well. All data analysis was completed using JMP Statistical Software by SAS.

Results

Germination and Establishment

The low treatment sections had two sections without any thistles present. The other two chambers had three thistles germinated and established out of the 12 original planting point, a 25% success rate. The medium treatment sections had one section without any thistles present. The other three chambers had a total of six thistles germinated and established a 50% success rate. In the low and medium treatment there was one thistle that germinated and died off within the first few weeks. The rest of the thistles germinated and survived. The high treatment sections all had thistles present, with a total of nine thistles germinating and establishing, a 75% success rate.

Soil Moisture and Thistle Performance

The medium water treatment had the highest amount of excess volumetric soil moisture content throughout the experiment (Figure 24). The high water treatment had the second highest amount of excess volumetric soil moisture content followed by the low treatment. The musk thistles in the high water treatment had the largest thistles, followed by the medium treatment and then the low treatment thistles (Figure 25).

Discussion

As I hypothesized, the musk thistle germination and establishment rate increased as the amount of water per treatment increased. The high water treatment, 1.1 liters, twice a week had the most seeds germinated and establish musk thistle plants followed by the medium treatment, 0.76 liters, and the low treatment, 0.38 liters. The low and medium water treatments each had one seedling die after it emerged. Therefore, it can be assumed that if musk thistle seeds have an adequate amount of water in the soil surrounding them, they will successfully germinate and establish in the field. Bare ground areas with musk thistle present in the seed bank or near a thistle patch that have moist soils for a week or longer are most vulnerable to a thistle establishment and should be monitored closely. Once the seedlings emerge, if they receive a consistent supply of water, there will be low mortality rates and large rosettes will form. In this study, as the amount of water increased per treatment, the average musk thistle rosette size increased, as I had hypothesized. In the field it can be assumed, during the early establishment phase, the more water present in the soil the larger the rosettes will grow early on, which can help aid their success.

Originally, I had hypothesized there would be less excess water in the high water treatment versus the others, because there would be more thistles utilizing the water. This did not prove to be true, as the medium treatment had the highest available water followed by the high and low treatments. The high and low treatments show similar volumetric soil moisture readings across the all the dates. This is likely caused by there being no thistles present in two sections for the low treatment, so no water would have been used in these sections, which could have skewed the results. This also occurred in

one section of the medium water treatment that had no thistles as well. The high water treatment sections had lower soil moisture readings than the medium treatment. This can be explained by the larger rosettes, larger tap roots, and more thistles present in the high water treatment, which would have been utilizing the water better than the medium treatment thistles.

There are several limitations that need to be addressed about this study. Due to the way the terrarium was set up, there was no drainage for the water, it accumulated at the bottom of the sections. This probably affected the soil moisture readings in the sections that had no thistles present, creating higher readings, which could have affected the overall trends for each treatment. Due to the lack of drainage, the soil stayed moist longer than it would out in the field which would affect how these results can be applied to management plans. The lack of drainage made it easier for musk thistles to have available water a lot longer than in the field, which could have affected the size of the rosettes and the success of the thistles. Future studies should include more replications per treatment type which may provide stronger statistical power to be able to analyze the impacts of water limitation on musk thistle success. More replications for each treatment can show if the establishment rates are consistent or if there were outside effects altering this study. It can also allow for management implications to be determined with stronger evidence supporting them.

Conclusion

Based on this study, bare ground or recently disturbed areas with no below ground root competition, available light, and soil nitrogen, and moist soils are the most vulnerable to a musk thistle establishment and should be monitored closely. In the field it is probable that if musk thistle is able to establish earlier than native species and utilize the available resources, such as water, earlier, then they will more successful than the natives. If musk thistle is able to establish a foothold in its local environment it can be extremely disruptive to emerging native species. During drought years or dry periods bare ground areas are less likely to be vulnerable to musk thistle establishment, as water seems to be a limiting factor in thistle success during the early establishment phase. All in all, the more soil moisture present around a musk thistle during the early establishment phase of its lifecycle, the more successful the thistle will be.

Literature Cited

Doing, H., E.F. Biddiscombe, and S. Knedlhans. 1969. Ecology and distribution of the Carduus nutans group (Nodding Thistles) in Australia. *Vegetation* 17(1):313–351.

Hamrick J.L., and Janet M. Lee. 1987. Effect of Soil Surface Topography and Litter Cover on the Germination, Survival and Growth of Musk Thistle (*Carduus nutans*). *American Journal of Botany* 74(3): 452-457.

Lee, J.M., and J.L. Hamrick. 1983. Demography of two natural populations of Musk Thistle (Caduus nutans). *Journal of Ecology* 71:923–936.

McCarty, M.K., C.J. Scifres, A.L. Smith, and G.L. Horst. 1969. Germination and early seedling development of Musk and Plumeless Thistles. *University of Nebraska*.

Peterson-Smith, J. and K. Shea. 2010. Seedling emergence and early survival of *Carduus* spp. In three habitats with press and pulse disturbances. *Journal of the Torrey Botanical Society* 137:287-296.

Ruggiero, D.C., K. Shea. 2011. Effects of Interspecific Competition on Early Life History of the Invasive Thistles *Carduus nutans* and *C. acathoides. Northeastern Naturalist* 18(2):197-206.

Figures

Figure 22: The photograph shows the terrarium as a whole.

Figure 23: The photograph shows one of the chambers and how it is divided into three different sections where the thistles were planted.

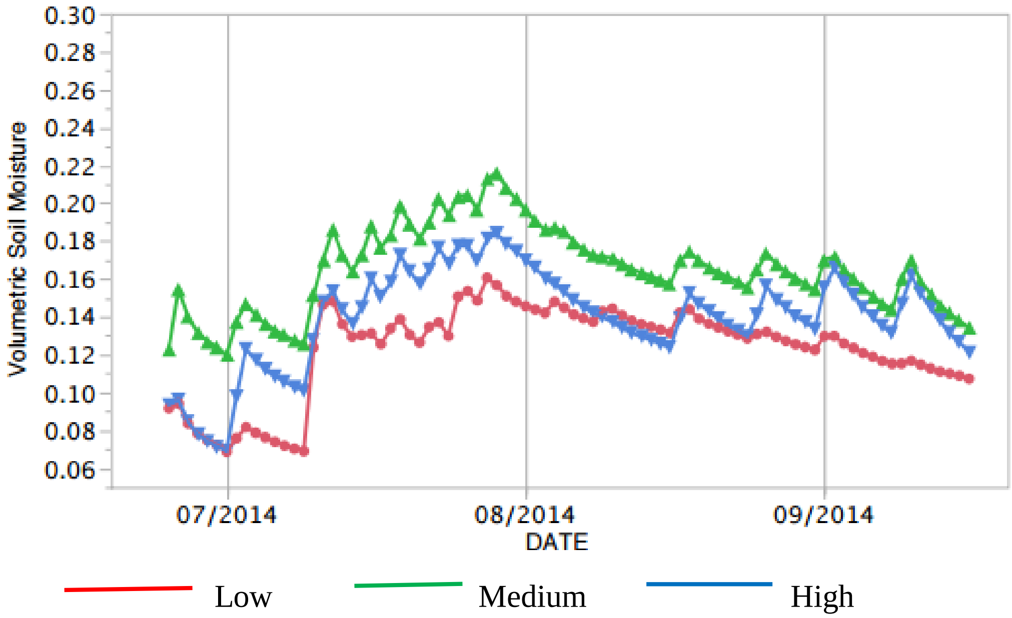


Figure 24: Average volumetric soil moisture for each treatment level by date.

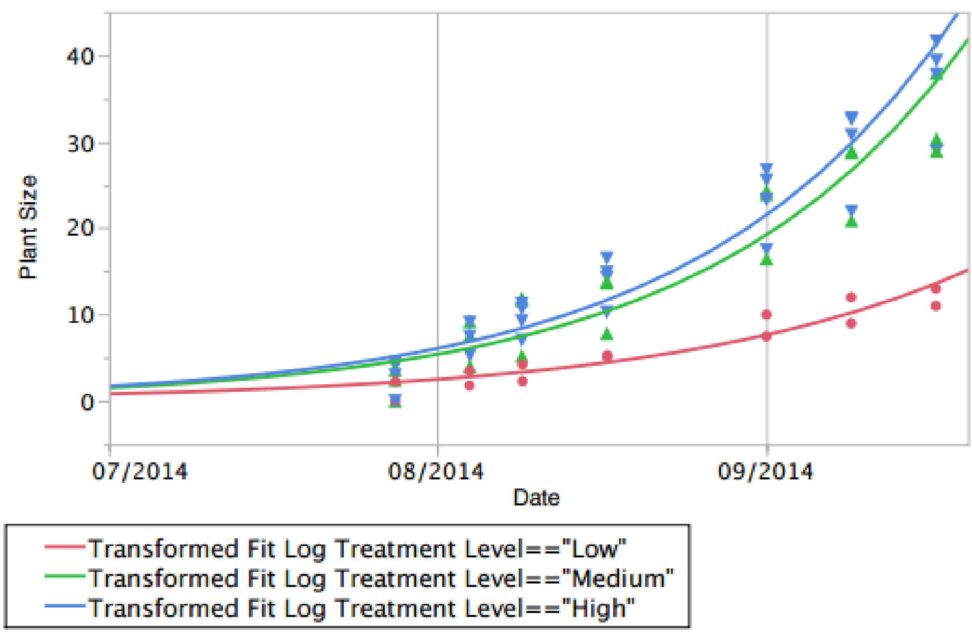


Figure 25: Log transformed average musk thistle rosette size for each treatment by date.