AMINOCYCLOPYRACHLOR EFFICACY ON NATIVE FORBS, AND SOIL SEEDBANK CHANGE 15 YEARS FOLLOWING RELEASE OF LEAFY SPURGE (*EUPHORBIA ESULA* L.) BIOLOGICAL CONTROL AGENTS

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Title

Aminocyclopyrachlor Efficacy on Native Forbs, and Soil Seedbank Change 15 Years Following Release of Leafy Spurge (*Euphorbia esula* L.) Biological Control Agents

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Native prairie response to aminocyclopyrachlor (AMCP) was evaluated in the Northern Great Plains. AMCP altered plant communities and reduced foliar cover of introduced, high seral, and low seral forb species. After treatment, high and low seral monocots increased at both sites due to reduced competition from susceptible forbs. AMCP reduced richness, evenness, and diversity. *Aphthona* spp. were released in the Little Missouri National Grasslands in North Dakota in 1999 for leafy spurge biological control. Soil seedbank composition was sampled from two ecological sites to evaluate changes in the plant community. By 2014, leafy spurge abundance decreased 92% on average. Subsequently, Kentucky bluegrass increased, but has also been deterred by a slow reintroduction of native species. High seral forb species richness has doubled in both ecological sites since 1999. *Aphthona* spp. successfully controlled leafy spurge for over 15 yr without any additional control methods or costs to land managers.

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OBJECTIVES

I. Determine effect of aminocyclopyrachlor (AMCP) on native forb species.

II. Determine the change in soil seedbank composition 15 yr after the release of *Aphthona* spp. flea beetles on leafy spurge (*Euphorbia esula* L.).

INTRODUCTION

AMCP Efficacy Study

Native prairie habitat has declined more than any other ecosystem in North America in the past 185 yr (Samson and Knopf 1994). In North Dakota, less than 20% of the native prairie remains (ND Parks and Rec Dept 2014). The Prairie Pothole Region (PPR) of North Dakota is composed primarily of short-, mixed-, and tallgrass prairie. Interspersed with isolated wetlands and river systems, the PPR has tremendous natural resource value; however, the region is also valuable for agricultural production (Gleason et al. 2008). Consequently, tillage associated with agriculture has caused a decline in native prairie and has fragmented remaining grassland tracts. Moreover, native prairie has been degraded by invasive, non-native species (Johnson et al. 1994).

One of the greatest threats to croplands, rangelands, aquatic areas, and wildlands in the United States are invasive plant species, which reduce the productivity and biological diversity of all ecosystems (Mullin et al. 2000). Natural wildlands depend on a diverse community of native plants to maintain stability and ecological function; nevertheless, throughout the last century, large numbers of invasive plants have become naturalized in the United States.

Aminocyclopyrachlor (AMCP), a newer auxin mimic herbicide, was developed to control invasive and noxious weeds in non-crop areas (Finkelstein et al. 2008). AMCP has a broad weed control spectrum and potential for use in turf, vegetation management, and pasture markets (Anonymous 2009). However, the effect of the chemical on native species has not been widely studied.

Seedbank Succession Study

Leafy spurge (*Euphorbia esula* L.) is an aggressive and tenacious perennial weed native to Europe and Asia (Dunn 1979). The plant was first found in North Dakota in 1909 (Hanson and Rudd 1933), and added to the state noxious weed list in 1935 (Anonymous 1963). Leafy spurge has a competitive advantage over native species because the plant produces an abundant amount of seed, emerges early in the spring, and has a widespread root system (Dunn 1979).

No single method can be used to control leafy spurge because the weed invades various soil and habitat types; furthermore, even successful integrated pest management systems will not eliminate viable leafy spurge seed (Foley 2004). Due to the ample seedbank, leafy spurge often reestablishes following otherwise successful chemical control programs (Bowes and Thomas 1978a, 1978b). Additionally, the seed is viable for up to 8 yr, which increases the time needed to successfully eliminate the weed (Bowes and Thomas 1978b). Herbicides are the most commonly used and successful control method for leafy spurge (Lym and Messersmith 1985); however, herbicide use is limited due to prohibition in environmentally sensitive areas and high chemical and application costs (Lym and Tober 1997). In environments where other methods are cost prohibitive, biological control methods have been used and provide an economically inexpensive solution (Lym 2005a). The most commonly used biological control agents, *Aphthona* spp. flea beetles, are native to Eurasia and were introduced to North America to control leafy spurge in the mid-1990s.

LITERATURE REVIEW

AMCP Efficacy Study

Distribution and Geographic Variation

Grasslands contain few trees or shrubs, have a mixture of non-graminoid herbaceous forbs, and are dominated by species of the Poaceae family (Anderson 2006). Grasslands once existed on every continent except Antarctica and covered approximately 46 million km^2 of the surface of the earth. However, native grasslands have been substantially altered by human activity, especially due to conversion to agriculture. In the United States, grasslands are considered critically endangered (i.e., have declined by more than 98%) (Noss et al. 1995).

Grasslands occur in North America under a vast range of climatic conditions and cover about 15% of the continent with a diverse assemblage of vegetation types (Risser et al. 1981). Early French explorers referred to the extensive grasslands of North America as *prairies*, which means meadow or field in French, and is a term still used today. 'Prairies' describe areas extending from desert grasslands of southwestern United States and northern and central Mexico to the northern mixed-grass prairies of the Canadian Provinces of Alberta, Saskatchewan, and Manitoba.

Traditionally, the grasslands of North America have been divided into three ecological sectors based on height of the native grasses, which is ultimately a function of annual precipitation. The divisions include the western shortgrass prairie (260 to 375 mm precipitation), the eastern tallgrass prairie (625 to 1200 mm precipitation), and a blend of the two, a mixed-grass prairie (375 to 625 mm precipitation) (Anderson 2006). Dominant species in shortgrass prairies are 0.3 to 0.5 m tall and include species such as buffalograss [*Bouteloua dactyloides* (Nutt.) J. T. Columbus], and grama grasses including, blue grama [*Bouteloua gracilis* (Willd. ex

Knuth) Lag. ex Griffiths], hairy grama (*Bouteloua hirsuta* Lag.), and sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.]. Dominant tallgrass species reach heights of 1.8 to 2.4 m and include Indiangrass [*Sorghastrum nutans* (L.) Nash], switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), and little bluestem [*Schizachyrium scoparium* (Michx.) Nash]. Dominant mixed-grass species are 0.8 to 1.2 m tall and include little bluestem, western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve], and green needlegrass [*Nasella viridula* (Trin.) Barkworth]. Within particular grassland regions, different prairie types result as functions of soil, aspect, slope, position and other factors (Corbett and Anderson 2006; Curtis 1971; Nelson and Anderson 1983; Umbanhower 1992). Soil moisture availability is a function of variation in soils and topographic features and is a primary factor causing changes in vegetation patterns. For example, in the mixed-grass prairie, many tallgrass species will occur in moist, depressed areas rather than upland sites.

Pleistocene History

Grasslands may have been present on the continent for 20 million yr (Axelrod 1985; Benedict et al. 1996; Risser et al. 1981); however, the Central Grassland is a relatively young landscape (Anderson 2006). Climate change and the continental ice sheet, which occurred during the Pleistocene epoch around 2.59 million to 11,700 years before present (YBP), caused the destruction of the mid-continent grassland and prevented replacement by other vegetation types. Most of the Central Grassland was still dominated by spruce (*Picea* spp.) and jack pine (*Pinus banksiana* Lamb.) forest, if not covered with glacial ice at the peak of Wisconsin glaciation, 18,000 YBP (Delcourt and Delcourt 1981). During the early Holocene epoch (10,000 YBP), oak (*Quercus* spp.) savannah or grasslands existed in much of the current Central Grassland, except the eastern-most extension into the Midwest, which was oak-hickory (*Quercus* spp.-*Carya* spp.) forest. In the eastern tallgrass prairie, savanna and prairie replaced oak-hickory forest during the Hypsithermal period (beginning around 8000 YBP and ending 5000 to 3500 YBP), which was a warm, dry period accompanied with increasing fire frequency (Anderson 1998; Baker et al. 1996; Delcourt and Delcourt 1981; King 1981; Winkler 1995, 1997; Winkler et al. 1986).

Climate

Grasslands occur in conditions where mean annual temperatures and precipitation vary from 0 to 30 C, and 1300 mm to as little as 200 mm annually, respectively (Oesterheld et al. 1999; Risser et al. 1981; Sauer 1950); as a result, no single climate characterizes grasslands. Distinguishing climatic features of grasslands include: periodic droughts, frequent fires, occurrence on landscapes that are level to gently rolling, and an abundance of grazing animals (Anderson 1982, 1990; Risser et al. 1981; Sauer 1950). Prairies are generally characterized by an arid, cold season lasting for about six months of the year, accompanied by a hot season, which is broken into a wet and dry portion with climatic variations occurring in cycles (Carpenter 1940). The presence of cyclical droughts is a critical factor determining the vegetation type in an area (apart from edaphic variation) and contributes toward preventing grasslands from becoming deciduous forests.

Three major air mass systems influence the climate of the grasslands in the Great Plains of North America: Polar, Gulf, and Mountain Pacific (Figure 1) (Borchert 1950; Bryson and Hare 1974; Risser et al. 1981). Within the grasslands, the Polar Air Mass influences vegetation patterns due to decreased snow cover and increasing temperatures from north to south (Risser et al. 1981). The Gulf and Mountain Pacific Air Masses determine the east to west variations. The Gulf Air Mass originates in the Gulf of Mexico and carries humid air northward into the eastern portion of the Great Plains. The warm, humid air encounters cooler air and generates moisture or convectional storms; consequently, the Gulf Air Mass is commonly associated with precipitation.

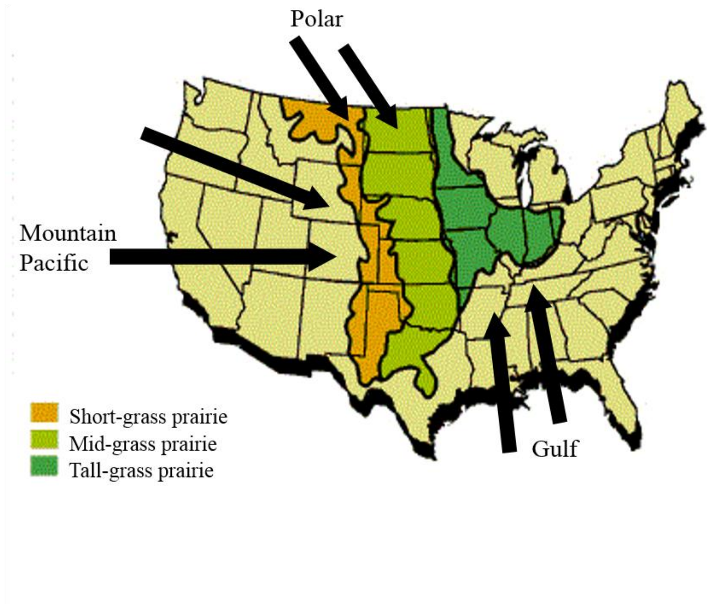

Figure 1. The extent of the Great Plains of North America and distributions of the major grasslands, and the air masses that influence the climate of the grasslands. Reillustrated from Borchert (1950), Risser et al. (1981), Anderson (1990), and Anderson (2006).

The Mountain Pacific Air Mass is also a humid air mass, but occurs on the west coast and progresses to the east, interacting with several mountain ranges such as the Coastal, Sierra Nevada, and Rocky Mountains (Risser et al. 1981). Thus, the grasslands lie within the rain shadow of the western mountain ranges. The air mass cools while rising, giving off much of the available moisture as orographic precipitation. As the air mass descends to lower elevations on the east side of the Rocky Mountains, the air is compressed by atmospheric pressure which causes warm, arid air to flow west into the Great Plains. From west to east, the frequency of the Pacific Air Mass decreases and the frequency of the Gulf Air Mass increases. Concurrently, periodic droughts, and periods of low humidity during summer decrease from west to east in the grassland (Borchert 1950; Bryson and Hare 1974; Risser et al. 1981). The west to east climatic variation causes changes in vegetation which characterize short-, mixed-, and tallgrass prairies (Anderson 2006).

Prairie Pothole Region (PPR)

The PPR of the Northern Great Plains extends from the north-central United States to south-central Canada and covers about 90 million ha (Gleason et al. 2008) (Figure 2). The PPR consists of three major physiographic regions of glacial origin, including: the Missouri coteau, the prairie coteau, and the glaciated plains (also known as drift prairie). Stagnant and dead-ice moraines formed the Missouri and prairie coteaus, resulting in a rugged area of closely spaced hills and depressions (Bluemle 2000). In contrast, the gently rolling landscape of the glaciated plains region was produced by ground-moraine processes (Gleason et al. 2008).

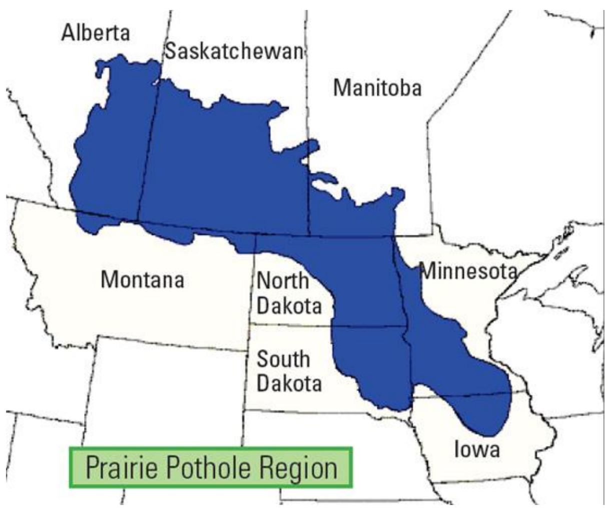

Figure 2. Extent of the Prairie Pothole Region in North America (USFWS 2016).

Historically, the PPR was composed primarily of short-, mixed-, and tallgrass prairie interspersed with numerous isolated wetlands and river systems (Gleason et al. 2008). The wetlands and riparian areas are important for wildlife habitat; however, the region is also valuable for agricultural production. Activities associated with agriculture have detrimental impacts on native prairie habitats (Tilman 1999). The primary cause of wetland loss is drainage to enhance agricultural production, while tillage has resulted in fragmentation, and a significant loss of native prairie. The few remaining tracts of native vegetation have become more susceptible to invasion due to fire suppression, changes in history, and introduction of exotic species (Gleason et al. 2008).

The US Department of Agriculture (USDA) has administered conservation programs to improve landscape conditions in the PPR. Approximately 2.2 million ha of PPR have been enrolled in either the Conservation Reserve Program (CRP) or Wetlands Reserve Program (WRP) (Gleason et al. 2008). The CRP provided long-term incentives for farmers to remove environmentally sensitive land from agricultural production and to plant species that will promote and improve environmental quality (USDA-FSA 2015). Similarly, the WRP provides technical and financial support to assist private landowners in establishing long-term conservation areas and practices (USDA-NRCS 2015). The long-term goal for conservation programs is to improve local and broad-scale environmental conditions including air and water quality, reduce hazardous risks by storing floodwater, provide increased recreational opportunities, and conserve biological resources (Allen and Vandever 2003; Gleason et al. 2008; Knutsen and Euliss 2001).

Native Prairie Plant Communities

Plant communities often contain dominant species that can overshadow other plants in both mass and biological activity (Whittaker 1965). However, many of the less abundant species are important for increased diversity and also contribute to overall community structure and function. Multiple hypotheses have suggested that community productivity and resource use as well as temporal stability depend on the number of species in a community (Tilman and Lehman

2001). Plant communities with greater diversity and species composition tend to be more stable and productive.

Native forbs are an essential component of prairie communities. Floristically diverse plant communities including more than 200 species of plants, with a majority being forbs, were once commonly found in the tallgrass prairie ecosystems of the Northern Great Plains (Beran et al. 1999; Jordan et al. 1988; Weaver 1954). Native forbs increase diversity (Hooper et al. 2005), increase aesthetic attributes, provide cover and seed for wildlife, and require low maintenance. Thus, interest in native forb seed production has increased (Weise et al. 2011) and forbs have become an important component of seed mixtures used for native species restoration in roadsides, parks, golf course roughs, and prairie restoration projects (Beran et al. 1999). A primary obstacle for the establishment of seeded prairie forbs and grasses is weed interference and competition (Dickens 1992; Howell and Kline 1993; Jacobs et al. 2007; Martin et al. 1982; Schramm 1978). The spatial and temporal distribution of native plants can slow the spread of invasion; yet invasive species often increase in population size and eliminate less abundant, less competitive native plants (Samuel and Lym 2008).

Nontarget Effects of Chemical Control

Establishment of invasive species can have devastating effects on native plant communities and natural wildlands. Herbage production of native species in wildlands, pasture, and range has been reduced 70 to 80% by leafy spurge infestations (Lym 2005b; Lym and Kirby 1987; Meiners et al. 2001; Selleck et al. 1962). In Theodore Roosevelt National Park, North Dakota, species richness in woodland communities was reduced up to 55% and several species that were consistently present in noninfested communities, were absent in leafy spurge-infested sites (Cogan and Butler 1999). Following chemical control of leafy spurge, perennial grasses increased on average from 41 to 98% of the plant composition in Montana; however, native forb production did not recover (Maxwell and Fay 1984).

Canada thistle [*Cirsium arvense* (L.) Scop.] has also been identified as a severe management problem in wilderness areas, national parks, and on The Nature Conservancy preserves (Randall 1996). Federal land management agencies such as the National Park Service (NPS) have utilized an integrated pest management program to control invasive species such as Canada thistle (Samuel and Lym 2008; USDI-NPS 1996). Broad-spectrum herbicides including picloram have been used in the past to control noxious and invasive weeds despite evidence of injury to nontarget (native) plant species (USDI-NPS 2007). For example, the use of picloram was only continued because state noxious weed laws required that Canada thistle be controlled, and there were no suitable alternatives (Samuel and Lym 2008). Following a fall application of aminopyralid, Canada thistle foliar cover and stem density decreased compared to the control. Aminopyralid reduced species richness and diversity in the native plant community 10 and 22 months after treatment (MAT), yet the relative abundances of the most common species were generally unchanged. Susceptible low and high seral forb species were reduced by aminopyralid.

Herbicides have been used to improve establishment of seeded native prairie species by reducing the adverse effects of weed interference (Beran et al. 1999); yet, the potential negative effects of herbicides on non-target, seeded species should be kept to a minimum. Atrazine and 2,4-D improved establishment of native grasses but had limited utility in the establishment of forbs (Cox and McCarthy 1958; Martin et al. 1982; Masters 1995). Forb establishment was greater following mowing than after applications of atrazine or 2,4-D when forbs and grasses were seeded together (Bragg and Sutherland 1989). Another study reported that no species of forb seedlings survived atrazine treatment, few survived sulfentrazone and oryzalin treatments, and the lowest seedling mortality was caused by dimethyl tetrachloroterephthalate (DCPA) and trifluralin (Jacobs et al. 2007).

Imazethapyr and imazapic have improved the establishment of some native forbs and can reduce weed interference in areas with high weed infestations (Beran et al. 1999). Perennial forb cover in some unburned areas increased following high application rates of imazapic (140 g ai ha^{-1} or greater) compared to burned areas; however, the pattern of perennial forb response to imazapic application rate was generally not consistent (Sheley et al. 2007). Annual forb cover response was less consistent than perennial forb response between herbicide rates and years. In the first year, annual forb cover was lowest in the plots with the highest rates of imazapic. Conversely, during the second year, annual forb cover was lowest in plots with the lowest rates of imazapic. Few consistent patterns relating the rate of imazapic to the cover of annual or perennial forbs have been observed (Monaco et al. 2005; Sheley et al. 2007).

Herbicides cannot be used in all areas where invasive species establish, such as ecologically sensitive areas, due to factors which include soil type and presence of endangered or threatened species. For example, the use of picloram is prohibited in the Sheyenne National Grassland (SNG) in North Dakota due to the sandy soil, a high water table, and the presence of the western prairie fringed orchid (*Platanthera praeclara* Sheviak and Bowles) (USFWS 1996). The orchid is a federally listed threatened plant native to the tallgrass prairie. Following loss of habitat, leafy spurge is the second largest threat to survival of the orchid (Lym 2005b; Sieg and Bjugstad 1994; USFWS 1996; Wolken et al. 2001). Consequently, the US Forest Service proposed implementation of a new integrated control program for invasive weeds at the SNG, which included quinclorac and aminocyclopyrachlor (Adams 2014).

Aminocyclopyrachlor

AMCP was recently developed for invasive and noxious weed control in pasture, range, and wildland (Finkelstein et al. 2008). AMCP is an auxin mimic herbicide (Turner et al. 2009), and is the first herbicide to be classified as a pyrimidinecarboxylic acid (Claus et al. 2010). The structure of AMCP (Figure 3) is similar to that of picloram, a pyridinecarboxylic acid herbicide used for broadleaf weed control in rangeland, pasture and non-crop areas (Oliveira et al. 2013). However, the chemical and physical properties of AMCP differ from those of picloram. For instance, the water solubility of AMCP and picloram are 4200 mg L^{-1} (Shaner 2014a) and 430 mg L^{-1} (Shaner 2014b), respectively, while soil sorption (K_{oc}) are 28 (Shaner 2014a) and 16, respectively (Shaner 2014b). Furthermore; AMCP half-life in Lamoure loamy sand averaged 40 d (Conklin and Lym 2013) compared to the average half-life of 90 d for picloram (Shaner 2014b). AMCP has both foliar and residual soil activity (Lindenmayer et al. 2010; Lym 2014; Sebastian et al. 2011; Westra et al. 2010).

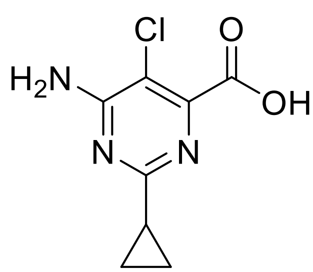

Figure 3. Chemical structure of aminocyclopyrachlor (Kegley et al. 2014).

AMCP will control many annual broadleaf weeds, as well as several invasive and woody plants. Susceptible weed species include absinth wormwood (*Artemisia absinthium* L.) (Conklin 2012; Endres et al. 2012), Canada thistle (Endres et al. 2012; Lindenmayer et al. 2010; Lym 2014; Vassios et al. 2010; Westra et al. 2010), and leafy spurge (Lindenmayer et al. 2010; Lym 2014; Westra et al. 2010). When applied at rates used to control annual broadleaf weeds, AMCP can injure some desirable forb species including sagebrush (*Artemesia* spp.) and flax species (*Linum* spp.) (Hergert et al. 2015). In general, all grass genera are more tolerant than broadleaf species to applications of AMCP, but variance for tolerance to AMCP exists within native plants.

Seedbank Succession Study

History

Leafy spurge was introduced from Eurasia to the United States through ship ballasts and seed stocks (Dunn 1985). The plant was first observed in the US in 1827 in Newbury, MA (Britton 1921). The weed was discovered in North Dakota in 1909, and by 1933, had become noted as serious problem infesting areas throughout the northern US spreading from Maine to Washington (Hanson and Rudd 1933). As of 2003, the species had been reported in 35 states and all but one Canadian province (Anderson et al. 2003). The dispersion of contaminated crop seed most likely caused the widespread distribution and continuous spread of leafy spurge (Messersmith and Lym 1983).

Biology

Leafy spurge is a dicotyledonous, herbaceous, deep-rooted perennial weed (Messersmith 1983). This species is one of the first to resume growth with emergence in the early spring (Bakke 1936). The first appearance of inflorescences varies between early and late May. Generally, the first viable seed is produced by late June and most of the seed matures during July; however, small amounts of seed can be produced through August and until frost (Messersmith 1983). The leaves are a characteristic bluish-green color and the bracts surrounding the reduced true flower parts are yellowish-green (Hanson and Rudd 1933).

Leafy spurge has a vigorous spreading root system, consisting of many coarse and fine roots, which act as an efficient storage organ (Hanson and Rudd 1933). This species can propagate sexually from seeds and asexually from root buds (Dunn 1979). Once established, leafy spurge spreads rapidly via roots and vegetative root buds. Roots of a single plant can spread up to 5 m in diameter per yr (Selleck et al. 1962). Insects are the main source of pollination and the plant rarely self-pollinates (Watson 1985). Leafy spurge plants consist of many flowering shoots and produce an average of 140 seeds per stem (Bakke 1936). When at maturity, leafy spurge seeds can be propelled up to 5 m (Bakke 1936), and can remain viable for up to 8 yr (Bowes and Thomas 1978b).

Leafy spurge stems are unbranched prior to flowering, erect, and fairly woody at the base; usually, several stems arise from the base (Hanson and Rudd 1933). The plant bears numerous linear-shaped leaves with smooth margins (Lym 1998). The leaves are entire or slightly sinuate, alternate, and whorl beneath the inflorescence (Watson 1985). When injured, the plant secretes a milky latex that can be toxic to livestock (Dunn 1979).

Economic Impact

Leafy spurge infestations have a negative impact on the economy (Lym and Messersmith 1983). In 2004, infestations on grazing land were estimated to result in a loss of regional grazing capacity sufficient to support a herd of 90,000 cows (Leistritz et al. 2004). Direct economic impacts on stock growers, landowners, and agribusiness firms were estimated to exceed $37 million annually, whereas secondary impacts throughout the regional economy reached almost $83 million. Impacts on wildland were smaller but still substantial. Additionally, reduced hay production and avoidance of grazing infested areas by cattle have a negative effect on the beef industry (Messersmith and Lym 1983).

Control Methods

Various methods have been used to manage leafy spurge including chemical, cultural, and biological control (Lym 1998). The most typically used herbicides include 2,4-D, dicamba, picloram, and quinclorac (Kuehl and Lym 1997; Lym and Messersmith 1985; USDA-ARS Team Leafy Spurge 2002). One of the reasons why leafy spurge cannot be eradicated with traditional control methods is the wide distribution of the weed and dispersed populations, which make applications of chemicals difficult and expensive (Dunn 1979). Also, many herbicides cannot be used in environmentally sensitive areas (Lym and Tober 1997). However, the wide range of environments where leafy spurge occurs is less of a deterrent to biological control agents (Dunn 1979).

Several biological control agents have been released to manage leafy spurge in the Northern Great Plains. The leafy spurge hawk moth (*Hyles euphorbiae* L.) was the first biological control agent, released in 1965 (Joshi and Olson 2009). The larvae feed on the leaves and flowers of leafy spurge; however, establishment has been relatively unsuccessful and the hawk moth has had minimal impact on the weed. In 1986, the leafy spurge gall midge (*Spurgia esulae* Gagne) was released. The larval stage of the gall midge feeds on terminal leaves and flower buds, which reduces the amount of seed production. The stem- and root-boring beetle (*Oberea erythrocephala* Shrank) was released in 1988. The larval stage of the beetle grows within the crown and stem, and the adults feed on leaves and flowers. However, the beetle has relatively low reproductive potential and has not reduced leafy spurge infestations (Julien and Griffiths 1999).

Leafy spurge flea beetles (*Aphthona* spp.) are the primary biological control agents released in North Dakota. *Aphthona* spp. are native to Eurasia and were introduced to the United

States in the mid-1980s (Anderson et al. 2003). In the Northern Great Plains, six different species have been introduced including: *Aphthona flava* Guill, *Aphthona cyparissias* Koch, *Aphthona czwalinae* Weise, *Aphthona lacertosa* Rosenhauer, *Aphthona abdominalis* Duftschmid and *Aphthona nigriscutis* Foudras (Carlson and Mundal 1990).

Flea beetle survival and establishment can depend on slope, soil type, density and type of surrounding vegetation, ground cover, temperature, and moisture (Gassman et al. 1996; Kirby et al. 2000). Of those released in North Dakota, *A. nigriscutis*, and *A. lacertosa* have been the most successful to establish (Lym 1998; Lym and Carlson 2002). Flea beetles provide control at the larval and adult stages; the larvae feed on the roots, and the adults feed on the above ground foliage (Joshi and Olson 2009). Although *Aphthona* spp. have become established at locations throughout the region, insect establishment and subsequent weed control has been low in areas where leafy spurge grows in sandy (> 80%) soil (Lym 2005a) or where periodically flooded. The poor establishment of *Aphthona* spp. on leafy spurge growing in sandy soil is likely due to the root system. Leafy spurge fine root structure is commonly found deeper in sandy than loamy soils, which may prevent the newly hatched larvae from finding a food source quickly enough to survive (Mundal and Carlson 1999).

Cultural control methods for leafy spurge such as grazing with angora goat (*Capra argagrus hircus*) have shown potential in sandy areas where other control methods have failed. Sheep and goat grazing can be economical and used in areas where herbicides cannot (Bangsund et al. 2001; Lym et al. 1997). Continuous grazing has considerably reduced leafy spurge densities and slowed the rate of spread through decreased amount of seed in the seedbank and reduced top growth (Bowes and Thomas 1978a). When sheep were removed from the grazed area, the weed regrew from the roots within 1 yr (Bowes and Thomas 1978a; Lym 2005a).

Sheep and goat grazing has successfully controlled leafy spurge infestations when combined with other methods such as herbicides (Lym 2005a; Lym et al. 1997).

Soil Seedbank

A soil seedbank consists of “all detached viable seeds of a species at a specific time” (Thompson and Grime 1979). On rangelands, seedbanks often occur as a thin, discontinuous layer and include seeds below, on, or near the soil surface (Young 1988). Baker (1989) added that a seedbank is an “aggregation of ungerminated seeds that have the potential to replace adult plants”. Thus, a seedbank is a product or “memory” of the past and represents the potential future of the aboveground plant community (Cavers 1995; Swanton and Booth 2004; Templeton and Levin 1979).

Soil seedbanks are determined by processes which affect outputs and inputs from local and long-range seed rain (Simpson et al. 1989). Outputs result primarily from seed germination, a process which is governed by genetically controlled physiological responses to environmental cues such as light, temperature, water, oxygen tension, and chemical stimulants. However, germination is not the only process that withdraws deposits from the seedbank (Baker 1989). Some seeds decompose or naturally lose viability (due to aging), others are consumed or destroyed by predation, and some are physically relocated. Factors such as density, species composition, and genetic diversity are directly controlled by input and output processes and indirectly influenced by life history processes (Simpson et al. 1989). Over time, seedbank dynamics are governed by shifts in the balance and relative importance of these processes that may vary between species and environmental conditions (Buhler et al. 1997).

Seedbanks of individual species can be classified as “transient” or “persistent” seed (Thompson and Grime 1979). Transient seed lasts for less than 1 yr, while persistent seed lasts for more than 1 yr and overlaps with fresh seed inputs each year. Persistent seeds tend to be small and compact, whereas short-lived seeds are generally larger and either flattened or elongated (Thompson et al. 1993). The relationship between seed size and shape and presence of a persistent seedbank is very likely correlated with the probability of burial. Except for self-burial techniques and ant dispersal, most mechanisms of burial (penetrating cracks in the soil, washing into soil from rain, ingestion by earthworms) are more likely to affect small, compact seeds compared to larger seeds. Moreover, some small seeds may also require burial for successful germination and seedling establishment (Peart 1984).

Seed persistence in the soil is not wholly determined by seed size and shape; other contributions include specific germination requirements, dormancy mechanisms, and resistance to pathogens (Thompson et al. 1993). Persistence also varies between species with different regeneration strategies (Buhler et al. 1997). Seeds of species that germinate shortly after they are spread generally have a short soil life; as a result, persistence is dependent on annual seed production and dispersal (Bazzaz 1996). In contrast, seeds of some species can be very long-lived and remain in the soil for extended periods with intermittent germination of part of the population (Buhler et al. 1997; Murdoch and Ellis 1992).

Seed persistence is a key plant characteristic and the largest component of many plant populations consists of buried seeds in the soil (Harper 1977). Moreover, seedbank dynamics determine the ability of a community to persist: a seedbank may contain diverse species and genotypes that could provide substantial flexibility for potential community changes (Leck et al. 1989). Information from soil seedbanks can play a fundamental role in the development of integrated pest management strategies by predicting potential weed emergence (Cardina and Sparrow 1996), as well as giving insights into management history by serving as a “memory” of the selective conditions that prevailed in the past and current conditions (Swanton and Booth 2004; Templeton and Levin 1979). The ability to predict the composition and density of weed seedling emergence allows land managers to reduce herbicide use, costs, and more efficiently implement control measures (Cardina and Sparrow 1996). Thus, an understanding of persistent seedbanks is crucial for informed vegetation management in agriculture and conservation (Thompson et al. 1993).

Knowledge of seedbanks becomes increasingly important when attempting to manage weeds within a framework of population and community dynamics to achieve long-term weed management. An important component of a long-term weed control strategy is management of weed seedbanks by targeting components of the plant life cycle directly by increasing seed mortality or manipulating germination and emergence, or indirectly by reducing seed production or removing above-ground vegetation (Swanton and Booth 2004). Jordan et al. (1995) suggested that management aimed at increasing seed mortality rather than solely killing weed seedlings was more effective because seed survival had a greater influence on population dynamics than plant survival or per plant seed production. The most efficient management strategy will depend on individual species traits, weed density, and environment, and must be geared toward the biology and ecology of the target species (Swanton and Booth 2004). The goal of management at the population level is to control a specific problem species; however, other species may be affected by the population control measure. The survival of many rare or declining species is dependent on management strategies that permit intermittent regeneration from a persistent seedbank (Keddy and Reznicek 1982; Lym 2005b).

Seedbank data can also be used for bioeconomic weed management models to estimate economic losses due to weeds and to evaluate management options (Schweizer et al. 1993;

Swinton and King 1994). The composition of the seedbank regulates important components (including timing and density of weed population), which affect the efficacy of control methods.

Evaluation of seedbank ecology is critical for 1) developing theories about plant community development, structure and function, 2) identifying factors regulating population dynamics, and 3) understanding successional patterns (especially following initial control of noxious weeds) (Perez et al. 1998). For example, Travnicek et al. (2005) determined that once Canada thistle was controlled, future site recovery was influenced by the amount of Canada thistle seed in comparison to desirable native species seed. Seedbank composition is a major contributor to vegetative regeneration patterns observed in disturbed areas (Perez et al. 1998).

Secondary Succession of Soil Seedbank

A leafy spurge soil seedbank study was initiated in the Little Missouri National Grasslands (LMNG) in western North Dakota in 1999. *Aphthona* spp. flea beetles were released in June at 12 loamy overflow, lowland and 12 loamy, upland sites. In the fall, soil samples were extracted from each site and the seedbank was evaluated. A total of 75 plant species germinated, and the most abundant species were leafy spurge, Kentucky bluegrass (*Poa pratensis* L.), prairie Junegrass [*Koeleria macrantha* (Ledeb.) Schult.], little bluestem, and green needlegrass (Cline 2002).

The study area was reevaluated in 2004, 5 yr after the release of *Aphthona* spp. flea beetles. Compared to 1999, leafy spurge top growth was decreased by approximately 90% via *Aphthona* spp. biological control agents. Leafy spurge composition in the seedbank decreased 86% in the loamy overflow and 92% in the loamy sites, respectively. Once again, the most abundant species were leafy spurge and Kentucky bluegrass (Juricek 2006).

Leafy spurge constituted only 2% of the loamy overflow seedbank and 15% of the loamy seedbank in 2009, 10 yr after the release of *Aphthona* spp., compared to 70 and 67% in 1999, respectively. Native species richness and seedbank seed count increased as leafy spurge abundance was reduced. High seral species represented 17% of the loamy overflow seedbank and 38% of the loamy seedbank in 2009, which increased from 5 and 13% in 1999, respectively. However, Kentucky bluegrass increased 220% in the loamy overflow and 650% in the loamy seedbanks. The reestablishment of native species had been slow, but seedbank analysis indicated the number and quality of species found prior to the leafy spurge infestation had increased (Setter and Lym 2013).

MATERIALS AND METHODS

AMCP Efficacy Study

The effect of aminocyclopyrachlor on the native plant community was evaluated at sites in Fargo, North Dakota and Felton, Minnesota. Neither site had been farmed or otherwise cultivated, and both supported diverse native flora. Fargo consisted of a mixed-grass composition while Felton was primarily composed of tall-grass prairie species. Both locations lie within the glaciated Lake Agassiz Plains region of the PPR (Gleason et al. 2008). The soil at the Fargo location was from the Fargo series which was 5% sand, 45% silt, and 50% clay with a pH of 7.2 and 7% organic matter. The Fargo location was classified as a clayey ecological site. Clayey ecological sites generally include species such as western wheatgrass, green needlegrass, porcupine grass (*Hesperostipa spartea* Trin.), American vetch (*Vicia americana* Muhl. ex Willd.), white sage (*Artemisia ludoviciana* Nutt.), white prairie aster (*Aster ericoides* L.), purple prairie clover (*Dalea purpurea* Vent.), and common yarrow (*Achillea millefolium* L.) (USDA-NRCS 2012). Extended nonuse will shift the plant community to one dominated by Kentucky bluegrass, smooth brome (*Bromus inermis* Leyss. ssp. *inermis)*, goldenrods (*Solidago* spp.), white sage, and western snowberry (*Symphoricarpos occidentalis* Hook.).

The soil in Felton was a Lohnes coarse sandy loam or Lohnes sandy loam consisting of 83.1% sand, 11.9% silt, and 5% clay, with a pH of 6.7, and 3.1% organic matter in the A horizon. The Felton location is classified either as a very shallow or sandy ecological site, with the majority of the area classified as very shallow (USDA-NRCS 2012). Very shallow ecological sites include plant species such as needle-and-thread [*Hesperostipa comata* (Trin. & Rupr.) Barkworth], blue grama, threadleaf sedge (*Carex filifolia* Nutt.), western wheatgrass, tarragon (*Artemisia dracunculus* L.), prairie coneflower [*Ratibida columnifera* (Nutt.) Woot. &

Standl.], fringed sage (*Artemisia frigida* Willd.), and prairie rose (*Rosa arkansana* Porter). Kentucky bluegrass and smooth brome will increase and dominate the plant community if present.

The study was a randomized complete block design (RCBD) with 9 replicates, treated and untreated, at each location. Each block was 9 m by 6 m and was divided into two plots of 4.5 m by 6 m. AMCP at 140 g ha^{-1} with a methylated seed oil (Upland MSO. West Central, Inc., 2700 Trott Ave SW, Willmar, MN 56201) plus silicone-based nonionic surfactant blend, Dyne-Amic (Helena Chemical Company, 225 Schilling Blvs, Suite 300, Collieville, TN 38017), at 0.25% v v^{-1} was applied in July to one random plot in each block with a hand-held boom sprayer equipped with four 8002 flat-fan nozzles (TeeJet, Spaying Systems Co. 200 W. North Ave, Glendale Heights, IL 60139) delivering 160 L ha^{-1} at 240 kPa.

Species composition was determined by visually assessing the plant foliar relative cover in four permanent 1-m^2 quadrats before treatment. Bare ground, litter, and individual plant species cover were estimated during peak standing biomass of cool season species in mid-June (before treatment) and of warm season species at the end of July (14 d after treatment). Evaluations before treatment and 14 d after treatement were indistinguishable and were combined and denoted as ‘0 months after treatment (MAT)’ in Tables 1 and 2. The plots were re-evaluated 10 and 14 months after treatment (MAT). Scientific nomenclature follows *Flora of the Great Plains* (Great Plains Flora Association 1986), except as amended by the USDA Plants Database (USDA-NRCS 2014). Plant species were separated into high and low seral floristic quality categories as defined by The Northern Great Plains Floristic Quality Assessment Panel (2001). Shrubby and woody plant species were minimal and were included in the forb categories. Change in vegetative components and plant species richness, evenness, and diversity estimated the effect of AMCP on the plant communities.

Seedbank Succession Study

The soil seedbank study was a continuation of previous research which was established in the LMNG in 1999 with 12 loamy overflow, lowland and 12 loamy, upland locations that were chosen based on leafy spurge density (uninfested, light, moderate, heavy), soil type, and vegetation composition. The LMNG is in an unglaciated section of the Northern Great Plains within the Badlands region in western North Dakota (Hanson and Whitman 1938) which has been seasonally grazed by cattle. The Badlands are characterized by striking differences in vegetation. Some of the vegetation types include: mixed prairie on uplands, little bluestem on hillsides, saltgrass [*Distichlis spicata* (L.) Greene] and sagebrush on stream terraces, prairie sandreed [*Calamovilfa longifolia* (Hook) Scribn.] on hills, and woodland along stream courses and ravines. The elevation at the sites ranges from 760 m at lowland, loamy overflow sites to 820 m at upland, loamy sites. The parent material consists of yellow and ash-gray shales, sandstones, clays, and scoria (which was produced by burning lignite beds) (Gauger et al. 1930). Erosion from wind, water, and burning lignite veins created topography with heterogeneity including plateau tops, slopes, terraces, valleys, buttes, low hills, and knobs of numerous shapes (Hanson and Whitman 1938).

Each site was marked with a global positioning unit, a PVC post, and two plastic surveyor stakes in 1999. The sites were 255 m^2 in size and were separated into eight equal transects radiating from the center at 45-degree angles. Leafy spurge density was estimated in June of 2014 by taking stand counts with 0.25-m^2 quadrats at 1-m intervals along transects in each cardinal direction. In August 2014, five transects were randomly assigned, and four soil cores were collected at 1-m intervals along each sampled transect. A total of 480 soil cores were excavated to a depth of 5 cm with a 10-cm diameter using a standard golf-cup cutter, as per the methods of Cline (2002).

Seedbank analysis was conducted by seed germination methods outlined by Ter Heerdt et al. (1996). Prior to germination, the soil samples were refrigerated at 3 C. Greenhouse trays, 28- by 56-cm, were prepared by adding a 1:1 mixture of steam-sterilized black sandy loam soil and commercial plant-growth media (Sunshine Mix No. 1, patented formulation with wetting agents. Sun Gro Horticulture, 770 Silver St., Agawam, MA 01001) to a depth of 2.5 cm. Next, the tray was topped with a 3- to 5-cm thick layer of sterile silica sand. Four soil cores from each transect were combined and washed through a coarse (4 mm) and fine (0.2 mm) sieve, and then added to the trays as the final layer. The trays were placed in the greenhouse, watered daily, and rotated weekly. Greenhouse temperature was maintained between 20 and 28 C, and natural light was supplemented with 450 $\mu E\ m^2\ s^{-1}$ to total 16 h day^{-1}.

Each species was identified as soon as possible after emergence, recorded, and removed. Coefficients of Conservatism (C-value) were assigned to each plant species based on an assessment by The Northern Great Plains Floristic Quality Assessment Panel (2001). The species were placed into seven plant categories which included the major invasives leafy spurge and Kentucky bluegrass, high seral forbs, low seral forbs, high seral grasses, low seral grasses, and hydric/mesic species (Cline 2002). High seral species (assigned a C-value of ≥ 4) are found in undisturbed and stable plant communities and generally indicate a high quality plant community. Low seral species (assingned a C-value of ≤ 3) are found in areas with high disturbance levels and indicate an early successional, low quality prairie (Juricek 2006). Introduced species were included in the low seral species categories and any shrubs or woody species were included in the forb categories. The identified seedlings were used as a proxy to determine the abundance of seed in the samples collected from the soil seedbank. The study ended 6 wk after the last seedling was removed from an individual tray (Ter Heerdt et al. 1996), or after 9 mo from the starting date of germination, whichever was earlier.

Data Analysis

The data for the efficacy of aminocyclopyrachlor study were analyzed as an RCBD. Changes in individual plant species percent foliar cover, richness (number of species present in plots), evenness (relative abundance of species within plots), and diversity between treated and untreated communities were analyzed using ANOVA in SAS (Statistical Analysis Software, Version 9.3. SAS Institute, Inc., 100 SAS Campus Dr., Cary, NC 27513). Plant community diversity was calculated using the Shannon-Wiener diversity index. Richness, evenness, and diversity indices for each plot were calculated using PC-Ord 6 (PC-ORD, Version 6. Multivariate Analysis of Ecological Data. MjM Software, PO Box 129, Gleneden Beach, OR, 97388), prior to the calculations, cover data were transformed using an arc sine square-root method.

The soil seedbank experiment was an RCBD. A factorial arrangement was used to compare seedling densities within each vegetation category in each ecological site. Data were analyzed using a generalized Linear Model (GLM) in SAS. Treatment means were separated using a pairwise t-test ($P \leq 0.05$) provided by the probability of difference (PDIFF) command of SAS. There were 12 replicates and five sub-samples for each ecological site which were analyzed separately.

RESULTS AND DISCUSSION

AMCP Efficacy Study

Plant communities at two native prairie sites were altered by AMCP (Tables 1 and 2). AMCP reduced or eliminated many forb species from both communities. Foliar cover of monocot species increased likely due to the reduced competition from susceptible forbs. These results are consistent with a similar Minnesota field study that was conducted with aminopyralid in which the major change in the treated plant communities was a decrease in high and low seral forbs and undesirable species (Almquist and Lym 2010).

Table 1. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Fargo, ND prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a].

		0 MAT		10 MAT		14 MAT	
Scientific name[b]	Common name	Trt	Ctrl	Trt	Ctrl	Trt	Ctrl
		% foliar cover[c]					
Introduced species[d]							
Bromus inermis Leyss.	Smooth brome	0.2	0	2.7	2.9	3.2	2.9
Cirsium arvense (L.) Scop.	Canada thistle	1.1	1.5	0.2*	1.7	1.0*	3.7
Elymus repens (L.) Gould	Quackgrass	4.6	5.1	0.2	0	-	-
Euphorbia esula L.	Leafy spurge	1.9*	3.9	1.0*	6.2	3*	10.0
Poa pratensis L.	Kentucky bluegrass	6.7	6.5	30.7*	14.6	39.1*	15.8
Rhamnus cathartica L.	Common buckthorn	0*	< 0.1	0	0.1	< 0.1	0.2
Schedonorus pratensis (Huds.) P. Beauv.	Meadow fescue	0.3	0.8	-	-	0.1	< 0.1
Subtotal[e]		14.8*	19.1	34.8*	25.5	46.3*	32.5
High seral forbs							
Anemone canadensis L.	Canadian anemone	0.1	0.1	0	0.1	< 0.1	0.1
Apocynum androsaemifolium L.	Spreading dogbane	0.1	0.3	0.1	0.2	0.7	1.1
Aster simplex Willd.	White panicled aster	1.8	1.1	0*	1.0	< 0.1*	2.8
Cornus sericea L. ssp. *sericea*	Redosier dogwood	0.1	0.1	0.1	< 0.1	0.1*	0

Table 1. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Fargo, ND prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a] (continued).

Scientific name[b]	Common name	0 MAT Trt	0 MAT Ctrl	10 MAT Trt	10 MAT Ctrl	14 MAT Trt	14 MAT Ctrl
		% foliar cover[c]					
Crataegus dissona Sarg.	Northern hawthorn	-	-	-	-	< 0.1	< 0.1
Galium borale L.	Northern bedstraw	5.7	4.8	0*	4.4	< 0.1*	4.3
Helianthus maximiliani Schrad.	Maximilian sunflower	0.3*	0	-	-	< 0.1	< 0.1
Lithospermum canescens (Michx.) Lehm.	Hoary puccoon	0.5	0.3	0.6	0.4	0.6	0.4
Lonicera dioica L.	Limber honeysuckle	0.2	< 0.1	< 0.1	0	0.1	< 0.1
Ribes ozyacanthoides L.	Canadian gooseberry	< 0.1	0.1	0.1*	0.2	0.1	0.2
Sisyrinchium angustifolium Mill.	Narrowleaf blue-eyed grass	-	-	-	-	0	< 0.1
Solidago canadensis L.	Canada goldenrod	6.7	5.1	0*	6.7	0.2*	8.1
Thalictrum dasycarpum Fisch. & Avé-Lall.	Purple meadowrue	3.3*	1.8	1.0*	2.4	0.9*	2.3
Vicia americana Muhl. ex Willd.	American vetch	0.6	0.5	< 0.1*	0.7	0.1*	0.7
Viola pedata L.	Birdfoot violet	0.5*	0.1	0*	0.2	0*	0.2
Subtotal[e]		19.8*	13.7	1.9*	16.2	2.9*	20.2
Low seral forbs							
Artemisia ludoviciana Nutt.	White sage	1.2*	0.2	0.3	0.4	0.8	0.4
Asclepias syriaca L.	Common milkweed	0.3*	0.8	0.1	0.2	0.2*	0.6
Aster ericoides L.	White prairie aster	2.6	2.1	0.4*	3.5	1.0*	4.0
Glycyrrhiza lepidota Pursh	American licorice	1.8	2.0	0.1*	0.8	1.5*	5.0
Juniperus virginiana L.	Eastern redcedar	0.1	< 0.1	0.1	0	0.1	< 0.1
Rosa arkansana Porter	Priarie rose	5.6	5.8	1.6*	5.1	5.3*	8.7
Symphoricarpos occidentalis Hook.	Western snowberry	10.2*	12.9	6.9*	11.5	7.4*	11.6
Taraxacum officinale F. H. Wigg	Common dandelion	0.3	< 0.1	0.1*	0.4	0.1*	0.4
Subtotal[e]		22.1*	27.1	9.6*	22.1	16.3*	30.7

Table 1. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Fargo, ND prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a] (continued).

		0 MAT		10 MAT		14 MAT	
Scientific name[b]	Common name	Trt	Ctrl	Trt	Ctrl	Trt	Ctrl
		% foliar cover[c]					
High seral monocots							
Carex pellita Muhl. ex Willd.	Woolly sedge	5.9	4.4	1.0	1.2	1.0	1.0
Dichanthelium oligosanthes (Schult.) Gould	Scribner rosette grass	0.4*	0.1	0.3	0.1	1.0	0.3
Hesperostipa spartea (Trin.) Barkworth	Porcupinegrass	2.7	2.4	4.5	3.1	6.6*	2.6
Subtotal[e]		9	6.9	5.8	4.3	8.6*	3.9
Low seral monocots							
Subtotal[e]		-	- -	-	-	-	-
Total foliar cover		65.7	66.8	52.1*	68.1	74.0*	87.3
Bare ground		0.3	0	0.7	0.1	-	-
Litter		34.0	33.2	47.2*	31.8	26.0*	12.7
Species richness		13	12	9*	13	11*	14
Species evenness		0.94*	0.93	0.85*	0.92	0.88*	0.93
Diversity index[f]		2.3	2.3	1.8*	2.3	2.1*	2.4

[a]Applied July 2014 with an methylated seed oil (Upland MSO. West Central, Inc., 2700 Trott Ave SW, Willmar, MN 56201) plus silicone-based nonionic surfactant (Dyne-Amic. Helena Chemical Company, 225 Schilling Blvd, Suite 300, Collierville, TN 38017) blend at 0.25% v v^{-1}.
[b]Scientific nomenclature follows the Flora of the Great Plains (Great Plains Flora Association 1986), except as amended according to the United States Department of Agriculture (USDA)/ Natural Resources Conservation Service (NRCS) Plants Database (2014). Plant categories determined by United States Geological Survey (USGS): Northern Great Plains Floristic Quality Assessment Panel.
[c]Difference ($p < 0.05$) of plant species foliar cover, bare ground, litter, species richness, species evenness, and diversity between aminocyclopyrachlor treated and control plots within evaluation date was indicated by (*). Foliar cover of (-) indicates species not present in any plot within evaluation period.
[d]Non-native (introduced) according to the USDA/NRCS Plants Database (2014).
[e]Total foliar cover within selected plant category.
[f]Species diversity is represented by the Shannon-Wiener diversity index.

Table 2. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Felton, MN prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a].

		0 MAT		10 MAT		14 MAT	
Scientific name[b]	Common name	Trt	Ctrl	Trt	Ctrl	Trt	Ctrl
		% foliar cover[c]					
Introduced species[d]							
Agrostis stolonifera L.	Creeping bentgrass	-	-	5.5	4.4	3.4	4.2
Asparagus officinalis L.	Garden asparagus	-	-	< 0.1	0	< 0.1	0.1
Bromus inermis Leyss.	Smooth brome	1.6	1.8	1.5*	5.3	1.1*	5.3
Calystegia sepium (L.) R. Br.	Hedge bindweed	0.2	0.3	0	0.2	0*	0.4
Elymus repens (L.) Gould	Quackgrass	2.9	3.2	-	-	-	-
Eragrostis cilianensis (All.) Vign. ex Janchen	Stinkgrass	< 0.1	< 0.1	-	-	-	-
Lactuca serriola L.	Prickly lettuce	0.1	0.1	0	0.1	0*	0.1
Medicago lupulina L.	Black medic	0.3	0.3	0*	0.1	0*	0.1
Melilotus officinalis (L.) Lam.	Yellow sweetclover	< 0.1	< 0.1	-	-	0	< 0.1
Poa pratensis L.	Kentucky bluegrass	12.2*	9.4	20.0*	10.6	22.0*	11.1
Phleum pratense L.	Timothy	0.1	< 0.1	-	-	0.5	0.1
Trifolium pratense L.	Red clover	0.1	0.1	0	0.1	0	0.1
Subtotal[e]		17.5	15.2	27.1*	20.8	27.1	21.4
High seral forb							
Allium textile A. Nelson & J.F. Macbr.	Textile onion	-	-	-	-	0	0.1
Anemone canadensis L.	Canadian anemone	0.7	1.0	0.2	1	0.3	0.9
Antennaria parvifolia Nutt.	Small-leaf pussytoes	0.6	0	-	-	-	-
Apocynum androsaemifolium L.	Spreading dogbane	0.3	0.3	< 0.1*	0.2	0.1	0.3
Arabis hirsuta (L.) Scop.	Hairy rockcress	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Aster simplex Willd.	White panicled aster	0.9	1.1	0*	0.9	0*	1.3
Castilleja coccinea (L.) Spreng	Scarlet Indian paintbrush	< 0.1	< 0.1	-	-	-	-
Cirsium flodmanii (Rydb.) Arthur	Flodman thistle	0.6	0.3	0*	0.3	0*	0.3

Table 2. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Felton, MN prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a] (continued).

		0 MAT		10 MAT		14 MAT	
Scientific name[b]	Common name	Trt	Ctrl	Trt	Ctrl	Trt	Ctrl
		% foliar cover[c]					
Dalea candida Michx. ex Willd.	White prairie clover	< 0.1	< 0.1	0	< 0.1	< 0.1	0.1
Dalea purpurea Vent.	Purple prairie clover	0.3	0.2	0*	0.2	< 0.1*	0.3
Equisetum arvense L.	Field horsetail	0.1	< 0.1	0.2*	0.1	0.1	0.1
Fragaria virginiana Duchesne	Wild strawberry	1.8	1.9	0.8*	2.0	0.8*	2.1
Fraxinus pennsylvanica Marshall	Green ash	0.1	0.3	0*	0.3	0*	0.4
Galium borale L.	Northern bedstraw	2.0*	4.6	0*	4.0	0.1*	4.2
Gentiana andrewsii Griseb.	Closed bottle gentian	< 0.1	0	0	0.1	0	< 0.1
Geum triflorum Pursh	Prairie smoke	0.2	0.1	0	0.1	< 0.1	0.1
Helianthus maximiliani Schrad.	Maximilian sunflower	0.9	0	-	-	0	0.1
Hypoxis hirsuta (L.) Coville	Yellow stargrass	0.2	0.1	0	< 0.1	0	< 0.1
Lithospermum canescens (Michx.) Lehm.	Hoary puccoon	0.1*	0.5	0*	0.4	< 0.1*	0.5
Lobelia spicata Lam.	Palespike lobelia	< 0.1	0.1	< 0.1	0	< 0.1*	0.1
Lysimachia ciliata L.	Fringed loosestrife	< 0.1	0	0	< 0.1	0	< 0.1
Lysimachia hybrida Michx.	Lowland yellow loosestrife	-	-	0.1	< 0.1	0.1	0.1
Oenothera rhombipetala Nutt. ex Torr. & A. Gray	Fourpoint evening primrose	0	< 0.1	0*	0.3	0*	0.3
Oligoneuron rigidum (L.) Small var. *rigidum*	Stiff goldenrod	1.8	2.3	0*	2.4	0*	2.6
Packera paupercula (Michx.) Á. Löve & D. Löve	Balsam groundsel	< 0.1	< 0.1	0.1	0.1	0.1	0.1
Pedicularis canadensis L.	Canadian lousewort	0	0.1	0	0.1	< 0.1	0.1
Polygonatum biflorum (Walter) Elliott	Smooth solomon seal	0.2*	0.8	0*	0.7	0*	0.7
Prunella vulgaris L.	Common selfheal	0.6*	0.2	0*	0.2	0*	0.2
Rudbeckia hirta L.	Blackeyed susan	0	< 0.1	-	-	0	< 0.1
Solidago canadensis L.	Canada goldenrod	2.5	1.5	< 0.1*	1.9	< 0.1*	2.7

Table 2. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Felton, MN prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a] (continued).

		0 MAT		10 MAT		14 MAT	
Scientific name[b]	Common name	Trt	Ctrl	Trt	Ctrl	Trt	Ctrl
		% foliar cover[c]					
Thalictrum dasycarpum Fisch. & Avé-Lall.	Purple meadowrue	0.7	0.7	0*	0.9	0*	0.7
Vicia americana Muhl. ex Willd.	American vetch	1.0	0.7	0*	0.7	< 0.1*	0.7
Viola pedata L.	Birdfoot violet	0.2	0.5	0*	0.5	0*	0.5
Zigadenus elegans Pursh	Deathcamas	< 0.1	< 0.1	< 0.1	< 0.1	0	< 0.1
Zizia aptera (A. Gray) Fernald	Meadow zizia	1.2	1.2	0.2*	1.3	0.4*	1.5
Zizia aurea (L.) W.D.J. Koch	Golden zizia	1.6*	3.9	0.1*	3.8	< 0.1*	3.0
Subtotal[e]		18.5*	22.4	1.6*	22.4	2.0*	24.1
Low seral forbs							
Acer negundo L.	Boxelder	0.2*	0.3	0.1*	0.2	0.1*	0.2
Achillea millefolium L.	Common yarrow	0.1	0.1	0*	0.1	0*	0.1
Artemisia ludoviciana Nutt.	White sage	0.4	0.4	0*	0.7	0*	0.8
Aster ericoides L.	White prairie aster	0.9	1.0	0*	0.6	0*	0.8
Cerastium arvense L.	Field chickweed	-	-	0	< 0.1	0	< 0.1
Erigeron annuus (L.) Pers.	Eastern daisy fleabane	< 0.1	0	< 0.1	0.1	< 0.1	0.2
Glycyrrhiza lepidota Pursh	American licorice	0.4	0.6	0	< 0.1	0.4*	1.3
Helianthus annuus L.	Common sunflower	< 0.1	0	0	< 0.1	0.1	0.3
Parthenocissus quinquefolia (L.) Planch.	Virgina creeper	0.2	0.1	0	0.1	0	0.2
Ratibida columnifera (Nutt.) Woot. & Standl.	Prairie coneflower	< 0.1	0	-	-	-	-
Rosa arkansana Porter	Prairie rose	2.6	1.2	0.3*	1.1	0.4*	1.3
Salix spp.	Willow	0.1	0.1	0	0.1	0*	0.2
Symphoricarpos occidentalis Hook.	Western snowberry	5.9	6.9	0.6*	6.4	0.6*	6.5
Taraxacum officinale F. H. Wigg	Common dandelion	0.8	0.9	0*	0.7	0*	0.5
Viola sororia Willd.	Common blue violet	0.4	0.4	0*	0.3	0*	0.2
Subtotal[e]		11.4	11.6	0.9*	10.4	1.5*	12.5

Table 2. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Felton, MN prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a] (continued).

Scientific name[b]	Common name	0 MAT Trt	0 MAT Ctrl	10 MAT Trt	10 MAT Ctrl	14 MAT Trt	14 MAT Ctrl
		% foliar cover[c]					
High seral monocots							
Andropogon gerardii Vitman	Big bluestem	4.5	3.1	9.4*	3.8	8.9	4.9
Bouteloua curtipendula (Michx.) Torr.	Sideoats grama	-	-	0.2	0	0.2	0.3
Carex inops L.H. Bailey ssp. *heliophila* (Mack.) Crins	Sun sedge	0.8*	1.4	1.6	1.9	2.2	3.2
Carex sartwellii Dewey	Sartwell sedge	0.1	< 0.1	< 0.1	0.2	0.4	0.3
Dichanthelium oligosanthes (Schult.) Gould	Scribner rosette grass	0.2	0.3	0*	0.2	< 0.1*	0.4
Eleocharis macrostachya Britton	Pale spikerush	< 0.1	< 0.1	0.8	< 0.1	0.8	0.1
Hierochloe odorata (L.) Beauv.	Sweetgrass	-	-	< 0.1	< 0.1	< 0.1	< 0.1
Juncus balticus Willd.	Baltic rush	0	< 0.1	0	0.1	0*	0.8
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	0.7	0.5	-	-	-	-
Nasella viridula (Trin.) Barkworth	Green needlegrass	4.2*	2.4	2.1	1.9	1.3	1.4
Panicum virgatum L.	Switchgrass	-	-	-	-	0.2	0.3
Schizachyrium scoparium (Michx.) Nash-Gould	Little bluestem	1.5	1.8	2.5	1.8	4.5	3.3
Sorghastrum nutans (L.) Nash	Indiangrass	9.6	7.7	14.0*	1.8	8.5	4.3
Subtotal[e]		21.5*	17.4	30.6*	11.8	27.1*	19.0
Low seral monocots							
Cyperus spp.	Flatsedge spp.	-	-	0.2	0.3	0.2	0.3
Elymus canadensis L.	Canada wildrye	0.1	< 0.1	< 0.1	0	0.2	0.2
Pascopyrum smithii (Rydb.) Á. Löve	Western wheatgrass	-	-	0.5	1.3	11.4*	1.8
Phalaris arundinacea L.	Reed canarygrass	< 0.1	0.1	< 0.1	0.1	0	0.1
Subtotal[e]		0.1	0.1	0.7	1.6	11.7*	2.3
Total foliar cover		68.9	66.8	60.5	67.0	69.5*	79.3
Bare ground		0	< 0.1	-	-	-	-
Litter		31.1	33.2	39.5	33	20.5	20.7

Table 2. Foliar cover of individual plant species and species richness, evenness, and diversity within the plant community in Felton, MN prior to [0 mo after treatment (MAT)], and 10 and 14 MAT with aminocyclopyrachlor at 140 g ha^{-1} [a] (continued).

		0 MAT		10 MAT		14 MAT	
Scientific name[b]	Common name	Trt	Ctrl	Trt	Ctrl	Trt	Ctrl
		% foliar cover[c]					
Species richness		16	17	7*	16	9*	19
Species evenness		0.93	0.94	0.88*	0.94	0.86*	0.94
Diversity index[f]		2.6	2.6	1.7*	2.6	1.8*	2.7

[a] Applied July 2014 with an methylated seed oil (Upland MSO. West Central, Inc., 2700 Trott Ave SW, Willmar, MN 56201) plus silicone-based nonionic surfactant (Dyne-Amic. Helena Chemical Company, 225 Schilling Blvd, Suite 300, Collierville, TN 38017) blend at 0.25% v v^{-1}.
[b]Scientific nomenclature follows the Flora of the Great Plains (Great Plains Flora Association 1986), except as amended according to the United States Department of Agriculture (USDA)/ Natural Resources Conservation Service (NRCS) Plants Database (2014). Plant categories were determined by the Northern Great Plains Floristic Quality Assessment Panel (2001).
[c]Difference ($p < 0.05$) of plant species foliar cover, bare ground, litter, species richness, species evenness, and diversity between aminocyclopyrachlor treated and control plots within evaluation date was indicated by (*). Foliar cover of (-) indicates species not present in any plot within evaluation period.
[d]Non-native (introduced) according to the USDA/NRCS Plants Database (2014).
[e]Total foliar cover within selected category.
[f]Species diversity is represented by the Shannon-Wiener diversity index.

Both locations had high floristic quality with more species of forbs than grasses prior to treatment (Tables 1 and 2). Invasive and noxious weeds, such as Canada thistle and leafy spurge, were only present in Fargo, ND. Moreover, in Fargo, there were 33 different species observed, whereas 80 species were recorded in Felton. The lack of noxious species and the difference in species richness (an average of 12.5 compared to 16.5, respectively) between the sites indicated that plant communities in Felton had a higher floral quality than Fargo prior to treatment with AMCP.

AMCP decreased foliar cover of introduced forbs at both locations, but most introduced grass species were unaffected (Tables 1 and 2). By 14 MAT at Fargo, foliar cover of both Canada thistle and leafy spurge increased in the control. Canada thistle foliar cover in the control increased from 1.5 to 3.7% 14 MAT and leafy spurge increased from 3.9 to 10%. AMCP reduced Canada thistle cover from 1.1 to 0.2% 10 MAT but cover returned to the initial level of

1% by 14 MAT. Similarly, leafy spurge cover was also reduced by AMCP from 1.9 to 1.0% 10 MAT, but increased to 3% by 14 MAT. Despite the recovery, foliar cover of both treated species was still lower compared to the control. In Felton, AMCP eliminated hedge bindweed [*Calystegia sepium* (L.) R. Br.], prickly lettuce (*Lactuca serriola* L.), and black medic (*Medicago lupulina* L.) from the treated plant community. Quackgrass [*Elymus repens* (L.) Gould] and stinkgrass [*Eragrostis cilianensis* (All.) Vign. ex Janchen] were absent but were also not observed in the controls. Yellow sweetclover [*Melilotus officinalis* (L.) Lam.] and red clover (*Trifolium pratense* L.) were also eliminated after treatment in Felton, but populations were so small that the treated community was still similar to the untreated. The removal of these introduced species could allow high seral forb or perennial grass species to benefit from decreased competition and thus increase the floristic quality and stability of the community. Kentucky bluegrass was the only introduced species in which foliar cover increased in treated plant communities at both locations. Following AMCP treatment, Kentucky bluegrass cover increased 14 MAT from 6.7 to 39.1% at Fargo, and from 12.2 to 22% at Felton. The increase in Kentucky bluegrass was likely due to decreased competition from AMCP susceptible species.

AMCP decreased total high seral forb cover at both locations (Tables 1 and 2). High seral forb cover was reduced from 19.8 to 2.9% in Fargo and 18.5 to 2% in Felton 14 MAT, respectively. Cover of high seral forbs in the control increased from 13.7 to 20.2% 14 MAT in Fargo, while in Felton, foliar cover of high seral forbs increased only slightly and averaged 23%. Birdfoot violet (*Viola pedata* L.), a native perennial that blooms mid- to late-spring and grows 7-10 cm tall (USDA-USFS 2016), was recorded prior to treatment, yet was no longer observed in treated areas at either location by 10 MAT (Tables 1 and 2). This species generally grows in well-drained black prairie soils and undisturbed, high-quality habitats. The presence of birdfoot violet indicates a high floristic quality at both sites prior to treatment, but the species did not recover from the disturbance of AMCP.

The floristic quality at both locations declined following AMCP treatment due to the loss and/or reduction of many high seral forb species (Tables 1 and 2). In addition to birdfoot violet, foliar cover of white panicled aster (*Aster simplex* Willd.), northern bedstraw (*Galium boreale* L.), Canada goldenrod (*Solidago canadensis* L.), purple meadowrue (*Thalictrum dasycarpum* Fisch. & Avé-Lall.), and American vetch (*Vicia americana* Muhl. ex Willd.) were also reduced by AMCP at both locations. Cover of treated white panicled aster decreased from 1.8 to < 0.1% 14 MAT in Fargo, while in Felton, white panicled aster was eliminated by AMCP 10 MAT and did not reappear. Similarly, purple meadowrue was reduced in Fargo and eliminated in Felton. American vetch was reduced 14 MAT from 0.6 to 0.1% in Fargo and 1.0 to < 0.1% cover in Felton. Canada goldenrod cover decreased from 6.7 to 0.2% and from 2.5 to < 0.1% 14 MAT in Fargo and Felton, respectively. These results are supported by a greenhouse study in which Canada goldenrod was considered 'susceptible' to AMCP applications of 35 to 105 g ha^{-1} (Carter 2016). In a similar field study, aminopyralid also reduced white panicled aster, Canada goldenrod, purple meadowrue, and American vetch 10 MAT (Almquist and Lym 2010). Furthermore, American vetch was considered susceptible to aminopyralid in a 2008 study (Samuel and Lym 2008).

Recovery from AMCP applications may be possible for some species. Although high seral species were reduced in Fargo and Felton, cover of some species increased slightly from 10 to 14 MAT (Tables 1 and 2). Several species injured by aminopyralid were able to recover by 22 MAT in a Minnesota field study (Almquist and Lym 2010). Prior to treatment, northern bedstraw cover was 5.7 and 2.0% in Fargo and Felton. The plant was not observed 10 MAT at either location (Tables 1 and 2), but by 14 MAT some plants recovered slightly at both sites. Similarly, Canada goldenrod cover in Fargo decreased from 6.7 to 0% 10 MAT and the species reappeared 14 MAT.

Several other high seral forb species were also observed at both sites but response to AMCP differed (Tables 1 and 2). These species included spreading dogbane (*Apocynum androsaemifolium* L.), Canadian anemone (*Anemone canadensis* L.), and hoary puccoon [*Lithospermum canescens* (Michx.) Lehm.]. Spreading dogbane cover was reduced by AMCP in Felton from 0.3 to < 0.1% 10 MAT, but was unchanged compared to the control 14 MAT in Felton and during every evaluation in Fargo. Hoary puccoon foliar cover was also similar to the control during every evaluation only in Fargo, while in Felton cover differed between treatments even prior to application of AMCP, and decreased from 0.1 to < 0.1% 14 MAT. Hoary puccoon occurs only in sunny, high quality habitats with rich prairie soils (PFAF 2016b); therefore, the reduction of other species may have created an open niche for hoary puccoon to survive after AMCP treatment.

Many high seral species that were only observed in Felton were also reduced or completely eliminated by AMCP (Table 2). Species that were eliminated included Flodman thistle [*Cirsium flodmanii* (Rydb.) Arthur], green ash (*Fraxinus pennsylvanica* Marshall), fourpoint evening primrose (*Oenothera rhombipetala* Nutt. ex Torr. & A. Gray), stiff goldenrod [*Oligoneuron rigidum* (L.) Small var. *rigidum*], smooth soloman seal [*Polygonatum biflorum* (Walter) Elliott], and common selfheal (*Prunella vulgaris* L.). High seral forb species that were only reduced by AMCP included purple prairie clover (*Dalea purpurea* Vent.), wild strawberry (*Fragaria virginiana* Duchesne), palespike lobelia (*Lobelia spicata* Lam.), meadow zizia [*Zizia aptera* (A. Gray) Fernald], and golden zizia [*Zizia aurea* (L.) W.D.J. Koch]. Aminopyralid applications also reduced purple prairie clover in a Minnesota field study (Almquist and Lym 2010). In a greenhouse study, purple prairie clover was injured an average of 53% 10 wk after an AMCP application at 35 to 105 g ha^{-1} and was considered to be 'moderately susceptible' (Carter 2016). Golden zizia was reduced by AMCP in Felton (Table 2), but was not affected by aminopyralid applications (Almquist and Lym 2010). Differences between studies could have been caused by the selectivity of the different herbicides, variation in ecological sites, and/or difference in plant/ community responses.

Some high seral forb cover at each location was similar between treatments (Tables 1 and 2). In Fargo by 14 MAT, the unchanged species included limber honeysuckle (*Lonicera dioica* L.) and Canadian gooseberry (*Ribes ozyacanthoides* L.). Northern hawthorn (*Cratageus sericea* L. ssp. *sericea*) and narrowleaf blue-eyed grass (*Sisyrinchium angustifolium* Mill.) were not observed prior to treatment but cover was similar between treatments at 10 and 14 MAT. In Felton, hairy rockcress [*Arabis hirsuta* (L.) Scop.], white prairie clover (*Dalea candida* Michcx. ex Willd.), prairie smoke (*Geum triflorum* Pursh), balsam groundsel [*Packera paupercula* (Michx.) Á. Löve & D. Löve], and Canadian lousewort (*Pedicularis canadensis* L.) cover was similar between treated and control after AMCP treatment. Lowland yellow loosestrife (*Lysimachia hybrid*a Michx.) was only observed at 10 and 14 MAT in Felton and cover was similar between treatments. Similarities between treatments were most likely because these species were low-growing or were seedlings protected from the herbicide by the overlying canopy, or were species which rarely occurred in the plant community.

AMCP reduced foliar cover of several low seral forb species at both locations but some species were able to recover by 14 MAT (Tables 1 and 2). Low seral forb foliar cover decreased 10 MAT with AMCP from 22.1 to almost 10% in Fargo and from approximately 11 to < 1% 10

MAT in Felton. By 14 MAT, low seral species in Fargo began to recover and almost doubled to 16.3% cover, while species in Felton recovered more slowly and only increased to 1.5%. At both locations, AMCP reduced white prairie aster (*Aster ericoides* L.), American licorice (*Glycyrrhiza lepidota* Pursh), prairie rose (*Rosa arkansana* Porter), western snowberry (*Symphoricarpos occidentalis* Hook.), and common dandelion (*Taraxacum officinale* F. H. Wigg). However, species responses and recovery varied between locations and species recovery occurred more slowly in Felton than Fargo. For example, prairie rose cover in Fargo decreased from 5.6 to 1.6% 10 MAT and almost completely recovered to 5.3% 14 MAT (still lower than the control), but in Felton, cover decreased from 2.6 to 0.3% 10 MAT and remained at 0.4% 14 MAT. AMCP eliminated white sage (*Artemisia ludoviciana* Nutt.) cover in Felton. In Fargo, cover of white sage was likely reduced 10 and 14 MAT but was not statistically different because there was more white sage in the plots to be treated than in the control at the beginning of the study. In Fargo, common dandelion was the only one of the reduced low seral forb species that didn’t begin to recover 14 MAT; whereas only American licorice recovered substantially in Felton, increasing from 0% 10 MAT to 0.4% 14 MAT. Conversely, common dandelion in an aminopyralid study recovered to near pretreatment levels by 22 MAT (Almquist and Lym 2010). In another study, American licorice was considered ‘susceptible’ to aminopyralid and western snowberry was unaffected by aminopyralid treatments (Samuel and Lym 2008). Prairie rose and American licorice field results were consistent with a greenhouse study in which these species were considered ‘moderately susceptible’ to AMCP (Carter 2016).

Common milkweed (*Asclepias syriaca* L.) cover in Fargo was lower compared to the control prior to treatment, was similar at 10 MAT, and was lower than control again at 14 MAT (Table 1). Eastern redcedar (*Juniperus virginiana* L.) cover was similar to the control during every evaluation in Fargo most likely due to the small sample size. In Felton, Eastern daisy fleabane [*Erigeron annuus* (L.) Pers.], common sunflower [*Helianthus annuus* (L.)], and Virginia creeper [*Parthenocissus quinquefolia* (L.) Planch.] were the only three low seral forb species which had similar cover between the treated and control at every evaluation, again likely due to the small sample size because injury to these species was observed (Table 2). Field chickweed (*Cerastium arvense* L.) was only observed in Felton after AMCP treatment with cover similar to the control and may have grown from seed or plants were too small to observe prior to treatment and may have increased because of the lack of competition from susceptible forb species. Boxelder (*Acer negundo* L.) was reduced by AMCP in Felton; while common yarrow (*Achillea millefolium* L.), willow (*Salix* spp.), and common blue violet (*Viola sororia* Willd.) were eliminated from the treated plant community.

Foliar cover of high seral monocots tended to decrease in Fargo from approximately 9 to 5.8% 10 MAT but nearly recovered to initial levels by 14 MAT (Table 1). Conversely, high seral monocot cover increased in Felton from approximately 22 to 27% 14 MAT but reached a maximum of almost 31% at 10 MAT (Table 2). The only high seral monocot species which was found at both locations was Scribner rosette grass [*Dichanthelium oligosanthes* (Schult.) Gould]. In Fargo, Scribner rosette grass cover was different between treatments prior to application of AMCP but similar after treatment; while in Felton, AMCP reduced cover from 0.2 to < 0.1% 14 MAT. Woolly sedge (*Carex pellita* Muhl. ex Willd.) was similar between treatments during every evaluation in Fargo, but cover decreased from an average of 5 to 1% 10 MAT in both the treated and control. The decline in woolly sedge cover in Fargo was offset by an increase in porcupine grass [*Hesperostipa spartea* (Trin.) Barkworth] from approximately 3 to almost 7% cover, 14 MAT.

The increase in high seral monocot species in Felton was primarily due to big bluestem and Indiangrass [*Sorghastrum nutans* (L.) Nash]. Big bluestem and Indiangrass cover increased from 4.5 and 9.6% to 9.4 and 14% 10 MAT, respectively, but cover of each was similar to the control by 14 MAT. Sun sedge [*Carex inops* L.H. Bailey ssp. *Heliophila* (Mack.) Crins] also increased in Felton. Foliar cover of sun sedge was initially lower in the treated compared to the control before AMCP application, but similar to the control by 10 MAT. Sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.], sweetgrass [*Hierochloe odorata* (L.) Beauv.], and switchgrass (*Panicum virgatum* L.) were not observed in Felton prior to treatment, but were recorded and had similar cover to the control by 14 MAT. Once again, the presence of these species after treatment was likely due to the decreased competition from susceptible species or germination from the seedbank. Little bluestem cover tended to increase in both treated and untreated communities. Additionally, Sartwell sedge (*Carex sartwellii* Dewey) and pale spikerush (*Eleocharis macrostachya* Britton) cover was similar to control during every evaluation in Felton. The reduction in forb competition provided a niche for grass species to establish and allowed the plant community to shift towards a grass-dominated landscape. The presence of native and perennial grasses in the plant community is important to maintain community stability and to provide resistance against invasion of weedy species (Tilman and Lehman 2001).

Low seral monocot species were not observed in Fargo, while low seral monocot cover in Felton increased from 0.1 to almost 12%, 14 MAT (Tables 1 and 2). Western wheatgrass was not found at Felton prior to treatment but foliar cover reached 11.4% by 14 MAT. A flatsedge spp. (*Cyperus* spp.) was also observed only after AMCP treatment and cover was simlar to the control. Canada wildrye (*Elymus canadensis* L.) and reed canarygrass (*Phalaris arundinacea* L.) were similar between both communities every evaluation.

Total foliar cover 14 MAT was lower in the treated communities than the non-treated at both locations, but litter cover differed by location (Tables 1 and 2). In Fargo and Felton, total foliar cover was 74 and almost 70% 14 MAT in the treated compared to approximately 87 and 79% in the untreated, respectively. Differences in foliar cover between treatments were caused by the loss of forb species after AMCP treatment. Litter in Fargo increased after treatment as total foliar cover decreased; however, in Felton, litter was similar to the untreated during every evaluation. Litter has been identified as one of multiple predictors for invasibility (Emery and Gross 2006). Litter could reduce light levels and create a physical barrier to seedling emergence and growth which would inhibit seedling establishment (Maret and Wilson 2005), or litter could retain soil moisture and protect seeds from extreme environmental conditions which would promote seedling establishment (Eckstein and Donath 2005). Multiple experiments have supported the findings that litter reduces, rather than promotes seedling establishment (Emery and Gross 2006; Foster 1999).

AMCP decreased species richness at both locations due to the elimination of susceptible species, but more species were removed from the plant community in Felton than in Fargo (Tables 1 and 2). In Fargo, species richness in treated areas decreased 31% 10 MAT and 15% 14 MAT compared to initial levels, whereas richness in Felton declined 56% 10 MAT and 44% 14 MAT. Species richness in Fargo was similar between treatments prior to application with 13 species in the treated and 12 in the control, respectively. By 10 and 14 MAT, AMCP reduced the number of species to 9 and 11 species in the treated, compared to 13 and 14 species in the control, respectively. In Felton, 14 MAT, richness also decreased from 16 to 9 species while the control increased slightly from 17 to 19 species. Birdfoot violet was eliminated from both locations, whereas species such as white panicled aster, Flodman thistle, Maximillian sunflower, stiff goldenrod, common selfheal, purple meadowrue, and yellow stargrass were eliminated from Felton only. Based on the theory of niche complementarity, species rich communities should be less susceptible to invasion due to competitive interactions (Tilman 1997); however, experimental studies have both supported (Knight and Reich 2005) and contradicted this theory (Emery and Gross 2006).

Species evenness was decreased at both locations because of the reduction in abundance of species such as prairie rose, western snowberry, and American licorice (Tables 1 and 2). In Fargo, prior to application of AMCP, species evenness was greater in the treated community with 0.94 compared to 0.93 in the control, evenness decreased to 0.88 by 14 MAT, compared to the control which was unchanged. In Felton, species evenness was similar prior to treatment, then decreased from 0.93 to 0.86 14 MAT, compared to the control which remained at 0.94 during every evaluation. The evenness at which species are distributed within a pasture may be important in reducing weed abundance; for example, species that are evenly distributed in space may use resources more equitably and produce a competitive environment that is difficult for weeds to invade (Lyons and Schwartz 2001; Tracy and Sanderson 2004; Wilsey and Polley 2002; Wilsey and Potvin 2000).

The Shannon-Wiener diversity index (H’) is a function of both species richness and evenness; as such, diversity in Fargo was also reduced by AMCP both 10 and 14 MAT (Table 1). Diversity declined to 1.8 10 MAT but then increased to 2.1 by 14 MAT to reflect the slight recovery of species richness and evenness. Species diversity in Felton followed the same trend and decreased from 2.6 to 1.7 10 MAT then rose to 1.8 14 MAT (Table 2). In pasture communities, weed abundance has been negatively related to the Shannon-Wiener index and forage diversity; however, weed abundance has been better explained by evenness rather than richness or Shannon-Wiener diversity index (Tracy and Sanderson 2004). Those results are contradictory to other studies which have observed a positive relationship between diversity and weed abundance (Planty-Tabacchi et al. 1996; Stohlgren et. al 1998); differences could be due to changes in soil fertility, soil disturbance, propagule supply, and/or proximity and abundance of nearby weedy species.

A decline in diversity and shift in dominant species following AMCP treatment could have beneficial and/or adverse effects for a plant community, depending on the initial quality and composition of the site. In an infested community, AMCP can help control unwanted species and shift the plant community towards a more grass-dominated community. By reducing invasive or undesirable species, diversity also decreases, but quality and stability of the plant community may improve overall. A study in an established native plant community determined that identity of dominant species affected local invasibility, and *Andropogon*-dominated communities were the least invasible (Emery and Gross 2006). Thus, a shift towards a grass-dominant community could lead to a more stable, less invasible community. However, in high quality native prairies with many desirable forb species, AMCP will likely reduce or eliminate forbs and decrease overall flora quality and diversity of the plant community. Based on the theory of fluctuating resource availability, susceptibility to invasion is determined by resource supply rate rather than diversity and a plant community becomes more susceptible to invasion with an increase in available resources (Davis et al. 2000).

Environments are more susceptible to invasion during the period immediately following a disturbance, such as an herbicide application, in which vegetation is damaged or destroyed, when resource use declines, and these resources become available to other species (Davis et al. 2000). In diverse, productive communities, the intense competition for resources may keep invasibility low; however, these communities may become susceptible to invasion if there are periodic disturbances. The disturbance of AMCP and reduction of susceptible forbs could create an open niche in the community with available resources for exotic species to invade and could disrupt the ecological function or structure of the plant community. Various forb species such as Canada goldenrod and purple prairie clover have been considered susceptible in field and greenhouse studies to AMCP applications and treatment should be avoided; whereas, species such as prairie rose and American licorice have been considered moderately susceptible and may recover following AMCP treatments, especially at low application rates (Carter 2016). Species identity and diversity play a large role in community dynamics regardless of contradictory generalizations regarding community invasibility and the associated relationships (Emery and Gross 2006). Injury to susceptible forbs should therefore be considered before applying AMCP.

Seedbank Succession Study

Leafy Spurge Stem Density

Leafy spurge stem density decreased 94% and 88% by 2014 in the loamy overflow and loamy sites, respectively, 15 yr following the release of *Aphthona* spp. (Table 3). The study was initiated with four different infestation levels: uninfested, light, moderate, and heavy; and had a mean stem density of 110 and 78 stems m^{-2} in 1999 in the loamy overflow and loamy sites, respectively (Cline et al. 2008). After 5 yr of biological control, the mean stem density decreased to 7 and 10 stems m^{-2} in the loamy overflow and loamy sites, respectively. By 2009, stem densities were similar among all infestation levels, regardless of original density (Setter and Lym 2013). The mean stem density remained at these low levels for the last 10 yr, with an average of 2 and 9 stems m^{-2} in 2009 and 7 and 9 stems m^{-2} in 2014 at the loamy overflow and loamy sites, respectively.

Table 3. Leafy spurge stem density across four infestation categories every 5 years from 1999 through 2014 in loamy overflow and loamy ecological sites in the Little Missouri National Grasslands in southwestern North Dakota.[ab]

Year/Initial leafy spurge density classification	Ecological site	
	Loamy overflow	Loamy
	stems m^{-2}	
1999		
Uninfested	0	0
Light	87	46
Moderate	127	83
Heavy	224	183
LSD (0.05)	12	11
2004		
Uninfested	5	3
Light	1	0
Moderate	16	16
Heavy	7	20
LSD (0.05)	10	9
2009		
Uninfested	3	9
Light	2	10
Moderate	2	4
Heavy	1	15
LSD (0.05)	NS	NS
2014		
Uninfested	6	13
Light	3	8
Moderate	4	2
Heavy	12	14
LSD (0.05)	6	8
1999 Mean	110	78
2004 Mean	7	10
2009 Mean	2	9
2014 Mean	7	9

[a]The 1999 and 2004 data were originally published by Cline et al. (2008) and are included for comparison.

[b]The 2009 data were originally published by Setter and Lym (2013) and are included for comparison.

Biological control of leafy spurge with *Aphthona* spp. successfully managed infestations in the LMNG in southwestern North Dakota for the past 15 yr (Table 3). The flea beetles reduced leafy spurge in areas of high infestation levels with as much vigor and success as areas with low levels of infestation in addition to dispersing to and establishing at sites where flea beetles were never released. Once the *Aphthona* spp. established, leafy spurge infestations were successfully managed without additional costs or use of other control methods such as herbicides. No other single control method has efficacy which has lasted for over 15 yr (Lym 2005a). *Aphthona* spp. flea beetles were both effective and an economically feasible option for leafy spurge control across large areas and in a variety of terrain. Similarly, studies throughout the northern Great Plains have reported a substantial reduction in leafy spurge infestations due to flea beetles (Butler et al. 2006; Kirby et al. 2000; Lesica and Hanna 2004; Lesica and Hanna 2009; Mico and Shay 2002).

Total Seedling Emergence

Seedlings that emerged during the study were used to determine the abundance of seed in the soil seedbank samples collected from each ecological site. The total number of seedlings increased 45% in the loamy overflow sites and 32% in the loamy sites throughout the past 15 yr (Table 4). In 1999, a total of 5,042 seedlings germinated in the loamy overflow sites, which increased to an average of 6,798 in 2004 and 2009, then increased again to 9,079 seedlings in 2014. Total seedling emergence in the loamy sites increased from 2,052 seedlings in 1999 to 3,008 seedlings in 2014. Compared to the loamy sites, the loamy overflow sites had a greater seedling emergence due to more optimal growing conditions which contributed to greater growth and seed production that more than doubled the number of emergent seedlings, a consistent trend throughout the 15 yr study (Cline et al. 2008; Setter and Lym 2013). The loamy overflow sites were at lower elevations, had more moisture available from surface runoff and subsurface water movement, higher organic matter content, and were likely more fertile than the loamy soils (Hanna et al. 1982; Malo and Worcester 1975; Wolf 1987). Although not present in the seedbank, some loamy sites had dense layers of creeping juniper (*Juniperus horizontalis* Moench), which may have prevented seeds from entering the seedpool.

Table 4. Total number of seedlings that emerged from each plant category and total number of species in four plant categories across all soil cores in loamy overflow and loamy ecological sites every 5 years from 1999 through 2014 in the Little Missouri National Grasslands in southwestern North Dakota.

Ecological site	1999[a]	2004[a]	2009[b]	2014	LSD (0.05)
			no. seedling[c]		
Loamy overflow					
Leafy spurge	3,358	1,135	127	136	246
Kentucky bluegrass	1,066	1,226	3,783	2,351	388
High seral forbs	165	1,627	119	2,128	248
Low seral forbs	297	2,460	1,010	2,809	130
High seral grasses	101	180	804	363	160
Low seral grasses	42	110	99	442	74
Hydric/Mesic	13	60	8	840	169
Unknown	0	0	16	10	5
Total	5,042	6,798	5,966	9,079	553
		no. species[d]			
Major invasives[e]	2	2	2	2	
High seral forbs	6	14	13	21	
Low seral forbs	22	26	23	41	
High seral grasses	7	6	7	13	
Low seral grasses	4	3	6	6	
Hydric/Mesic/Unk[f]	2	3	6	11	
Total	43	54	57	94	

Table 4. Total number of seedlings that emerged from each plant category and total number of species in four plant categories across all soil cores in loamy overflow and loamy ecological sites every 5 years from 1999 through 2014 in the Little Missouri National Grasslands in southwestern North Dakota (continued).

Ecological site	1999[a]	2004[a]	2009[b]	2014	LSD (0.05)
	no. seedling[c]				
Loamy					
Leafy spurge	1,429	299	146	184	158
Kentucky bluegrass	160	182	99	1,095	365
High seral forbs	89	730	209	616	124
Low seral forbs	180	1,314	333	815	243
High seral grasses	168	213	163	209	43
Low seral grasses	6	5	4	42	13
Hydric/Mesic	20	43	10	35	8
Unknown	0	0	13	12	5
Total	2,052	2,788	977	3,008	703
	no. species[d]				
Major invasives[e]	2	2	2	2	
High seral forbs	7	13	13	14	
Low seral forbs	18	21	20	33	
High seral grasses	8	9	8	10	
Low seral grasses	3	2	2	6	
Hydric/Mesic/Unk[f]	2	4	9	7	
Total	40	51	54	72	

[a]The 1999 and 2004 data were originally published by Cline et al. (2008) and are included for comparison.
[b]The 2009 data were originally published by Setter and Lym (2013) and are included for comparison.
[c]Total number of seedlings that emerged per 0.5 m^2 from soil samples collected to a depth of 5 cm.
[d]Total number of species that emerged each year in each ecological site.
[e]Major invasives includes only leafy spurge and Kentucky bluegrass plant categories.
[f]Hydric/Mesic and Unknown plant categories were combined due to small size.

Seedbank studies vary widely in both seed density and the number of species present (richness); but, in general, native weedy species (low seral species) are characterized by seedbanks with a relatively large number of seeds (Rice 1989). Although dominant in the vegetation, grasses are relatively underrepresented in the seed bank compared to forbs which are the major contributors to seed pools and generally have higher species diversity and richness than grasses. Similar trends were observed in both loamy and loamy overflow ecological sites, every year.

The rise in total number of seedlings at both ecological sites can be partially attributed to the increase in species richness (number of species) which has doubled since 1999 (Table 4). In 2014 in the loamy overflow sites, 94 species emerged compared to 43, 54, and 57 species in 1999, 2004, and 2009, respectively. Species richness in the loamy sites also increased steadily from 39 species to 72 species by 2014. Increased species richness will increase biodiversity by introducing more genetic traits into the community (Tilman and Lehman 2001). Moreover, greater species diversity could potentially lead to increased stability and productivity due to competitive mechanisms such as efficient resource use and capture. More diverse communities tend to be more stable, even if individual species within the communities tend to be less stable (Tilman et al. 2001).

High seral forb richness increased to 21 species by 2014 (more than 3 times the number of species in 1999) in the loamy overflow sites, and doubled to 14 species in the loamy sites; whereas, the number of low seral forb species doubled in both ecological sites (Table 4). Forbs are important components of rangeland communities and provide much of the ecological and botanical diversity (McArthur 1988). Forbs are also valuable for providing palatable forage (with preferential palatability for some animals and species), increasing forage nutrient value (especially in the spring), increasing nitrogen fixation (from leguminous forbs), controlling erosion with ground cover, providing a low maintenance aesthetically pleasing landscape, and resisting the spread of wildfire. The number of high seral grass species also doubled by 2014 in the loamy overflow sites, but remained unchanged in the loamy sites. The number of low seral grass species increased at both sites but reached a maximum of only 6 species by 2014. In general, an increase in high seral species abundance in the seedbank indicates a trend towards a more natural and desirable plant community, and low seral species are still more desirable than invasive species and may play an important role in the function of the plant community.

Loamy Overflow Seedbank

Leafy spurge emergence from seed in the loamy overflow seedbank decreased 96% from constituting 67% of the seedbank with 3,358 seedlings in 1999 to 127 and 136 seedlings in 2009 and 2014, respectively (Table 4), constituting only 1.5% of the seedbank (Table 5). Another major invasive, Kentucky bluegrass, increased from 1,066 to 3,783 seedlings in 1999 and 2009, respectively, and then declined to 2,351 seedlings in 2014 (Table 4), constituting approximately 26% of the seedbank (Table 5). Across years, Kentucky bluegrass composition at approximately 26% still didn't completely replace the presence of leafy spurge in the seedbank, likely due to the decline from 2009 to 2014 and an increase in the number of other plant species. Kentucky bluegrass seed production has most likely been slowed by the reintroduction of native species into the seedbank.

Table 5. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy overflow sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999.

			2014	
Scientific name[a]	Common name	C-value[b]	No.[c]	%[d]
Major invasives				
Euphorbia esula L.	Leafy spurge	NV	136	1.50
Poa pratensis L.	Kentucky bluegrass	NV	2,351	25.89
Subtotal			2,487	27.39
High seral forbs				
Androsace occidentalis Pursh	Western rockjasmine	5	866	9.54
Antennaria neglecta Greene	Field pussytoes	5	1	0.01
Arabis spp.	Rockcress species	5-7	838	9.23
Artemisia frigida Willd.	Fringed sage	4	212	2.34

Table 5. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy overflow sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999 (continued).

			2014	
Scientific name[a]	Common name	C-value[b]	No.[c]	%[d]
Astragalus missouriensis Nutt.	Missouri milkvetch	7	9	0.10
Campanula rotundifolia L.	Harebell	7	4	0.04
Chamerion angustifoilum (L.) Holb.	Fireweed	5	1	0.01
Cirsium flodmanii (Rydb.) Arthur	Flodman's thistle	5	2	0.02
Comandra umbellata (L.) Nutt.	Bastard toadflax	8	17	0.19
Dasiphora fruticosa (L.) Rydb.	Shrubby cinquefoil	5	8	0.09
Endolepis dioica (Nutt.) Standl.	Suckley's endolepis	4	2	0.02
Ericameria nauseosa (Pall. ex Pursh) G.L. Nesom & Baird	Rubber rabbitbrush	4	7	0.08
Galium boreale L.	Northern bedstraw	4	40	0.44
Lesquerella arenosa var. *arenosa* (Richardson) Rydb.	Great Plains bladderpod	6	82	0.90
Linum perenne Pursh var. lewisii	Prairie flax	6	15	0.17
Lithospermum canescens (Michx.) Lehm.	Hoary puccoon	7	9	0.10
Lycopus americanus Muhl. ex W.P.C. Barton	American water horehound	4	1	0.01
Lysimachia ciliata L.	Fringed loosestrife	6	2	0.02
Mondarda fistulosa L.	Wild bergamot	5	4	0.04
Oenothera caespitosa Nutt. ssp. *caespitosa*	Tufted evening primrose	8	5	0.06
Physaria brassicoides Rydb.	Double twinpod	8	3	0.03
Subtotal			2,128	23.44
Low seral forbs				
Achillea millefolium L.	Common yarrow	3	181	1.99
Amaranthus retroflexus L.	Redroot amaranth	0	1	0.01
Ambrosia artemisiifolia L.	Common ragweed	0	16	0.18
Artemisia ludoviciana Nutt.	White sage	3	130	1.43
Cerastium arvense L.	Prairie chickweed	2	30	0.33
Chamaesyce serpyllifolia (Pers.) Small	Thymeleaf sandmat	0	37	0.41
Chenopodium album L.	Common lambsquarters	NV	3	0.03
Chenopodium glaucum L.	Oak-leaved goosefoot	NV	2	0.02
Cirsium arvense (L.) Scop.	Canada thistle	NV	2	0.02

Table 5. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy overflow sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999 (continued).

Scientific name[a]	Common name	C-value[b]	2014 No.[c]	2014 %[d]
Conyza canadensis (L.) Cronq.	Horseweed	0	10	0.11
Descurainia sophia (L.) Webb ex Prantl	Flixweed	NV	124	1.37
Draba nemorosa L.	Yellow whitlowort	1	238	2.62
Draba reptans (Lam.) Fernald	Carolina whitlowort	1	836	9.21
Epilobium ciliatum Raf.	Willowherb	3	95	1.05
Erigeron annuus (L.) Pers.	Daisy fleabane	2	4	0.04
Euphorbia prostrata Ait.	Prostrate spurge	1	50	0.55
Galium aparine L.	Stickywilly	0	9	0.10
Glycyrrhiza lepidota Pursh	American licorice	2	3	0.03
Grindelia squarrosa (Pursh) Dun.	Curly-cup gumweed	1	1	0.01
Hedeoma hispida Pursh	Rough false pennyroyal	NV	504	5.55
Lactuca serriola L.	Prickly lettuce	NV	1	0.01
Lappula occidentalis (S. Watson) Greene var. *cupulata* (A. Gray) Higgins	Flatspine stickseed	2	13	0.14
Lepidium densiflorum Schrad.	Greenflower peppergrass	0	51	0.56
Medicago lupulina L.	Black medic	NV	1	0.01
Melilotus officinalis (L.) Lam.	Yellow sweet clover	NV	9	0.10
Nepeta cataria L.	Catnip	NV	4	0.04
Oenothera laciniata Hill	Cutleaf evening primrose	1	8	0.09
Plantago patagonica Jacq.	Woolly plantain	1	61	0.67
Polygonum aviculare L.	Prostrate knotweed	0	4	0.04
Populus deltoides Bartr. ex Marsh ssp. m*onilifera* (Ait.) Eckenw.	Plains cottonwood	3	2	0.02
Potentilla norvegica L.	Norwegian cinquefoil	0	129	1.42
Ranunculus abortivus L.	Littleleaf buttercup	2	5	0.06
Ratibida columnifera (Nutt.) Woot. & Standl.	Prairie coneflower	3	35	0.39
Silene noctiflora L.	Night-flowering catchfly	NV	14	0.15
Solidago canadensis L.	Canada goldenrod	1	1	0.01
Symphyotrichum ericoides (L.) G.L. Nesom var. *ericoides*	White prairie aster	2	17	0.19
Taraxacum officinale F. H. Wigg	Common dandelion	NV	44	0.48

Table 5. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy overflow sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999 (continued).

Scientific name[a]	Common name	C-value[b]	2014 No.[c]	2014 %[d]
Thlaspi arvense L.	Field pennycress	NV	1	0.01
Tragopogon dubius Scop.	Yellow salsify	NV	13	0.14
Verbena bracteata Cav. ex lag. & Rodr.	Bigbract verbena	0	71	0.78
Veronia peregrina L.	Neckweed	0	49	0.54
Subtotal			2,809	30.94
High seral grasses				
Bouteloua curtipendula (Michx.) Torr.	Sideoats grama	5	1	0.01
Bouteloua dactyloides (Nutt.) J.T. Columbus	Buffalograss	4	19	0.21
Calamovilfa longifolia (Hook) Scribn.	Prairie sandreed	5	4	0.04
Elymus trachycaulus (Link) Gould ex Shinners	Slender wheatgrass	6	4	0.04
Hesperostipa comata (Trin. & Rupr.) Barkworth	Needle-and-thread	6	6	0.07
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	7	172	1.89
*Muhlenbergia racemosa (*Michx.) Britton, Sterns, & Poggenb.	Marsh muhly	4	3	0.03
Muhlenbergia richardsonis (Trin.) Rydb.	Mat muhly	10	13	0.14
Nassella viridula (Trin.) Barkworth	Green needlegrass	5	44	0.48
Pascopyrum smithii (Rydb.) Á. Löve	Western wheatgrass	4	26	0.29
Schizachyrium scoparium (Michx.) Nash-Gould	Little bluestem	6	67	0.74
Spartina pectinata Bosc ex Link	Prairie cordgrass	5	1	0.01
Sporobolus cryptandrus (Torr.) A. Gray	Sand dropseed	6	3	0.03
Subtotal			363	4.00
Low seral grasses				
Agropyron cristatum (L.) Gaertn	Crested wheatgrass	NV	7	0.08
Bromus arvensis L.	Japanese brome	NV	354	3.90

Table 5. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy overflow sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999 (continued).

			2014	
Scientific name[a]	Common name	C-value[b]	No.[c]	%[d]
Bromus inermis Leyss. ssp. *inermis*	Smooth brome	NV	43	0.47
Elymus repens (L.) Gould	Quackgrass	NV	2	0.02
Panicum capillare L.	Witchgrass	0	1	0.01
Poa compressa L.	Canada bluegrass	NV	35	0.39
Subtotal			442	4.87
Hydric/Mesic species				
Carex spp. L.	Sedges	NV	33	0.36
Cyperus odoeratus L.	Fragrant flatsedge	2	2	0.02
Eleocharis acicularis (L.) Roem. & Schult.	Needle spikerush	3	784	8.64
Juncus spp. L.	Rushes	NV	3	0.03
Typha spp. L.	Cattails	NV	18	0.20
Subtotal			840	9.25
Unknown species				
Unknown spp. 1 - 6		NV	10	0.11
Subtotal			10	0.11
Total			9,079	100

[a]Scientific nomenclature follows the Flora of the Great Plains (Great Plains Flora Association 1986), except as amended according to the United States Department of Agriculture (USDA)/Natural Resources Conservation Service (NRCS) Plants Database (2014).
[b]Coefficient of Conservatism (C-value) was assigned to plant species based on an assessment by the Northern Great Plains Floristic Quality Assessment Panel (2001). A coefficient value of 'NV' (no value) indicates an introduced or unidentified species; 0-3 indicates species that flourish in highly disturbed habitats; and values of 4-10 indicate species from less disturbed, natural areas.
[c]Total number of seedlings that emerged per 0.5 m^2 from soil samples collected to a depth of 5 cm.
[d]Percentage of total seedlings that emerged across all soil cores.

The number of high seral forb seed in the loamy overflow sites varied between years (Table 4). High seral forb emergence was similar in 1999 and 2009 with 165 and 119 seedlings, respectively, and increased in 2004 and 2014 to 1,627 and 2,128 seedlings, respectively. Western rockjasmine (*Androsace occidentalis* Pursh) and fringed sage (*Artemisia frigida* Willd.) were present every year and constituted an average of 3 and 5%, respectively, across all years in the loamy overflow sites (Table 5) (Setter and Lym 2013). In 2004, four species primarily contributed the overall increase in high seral forb composition (Cline et al. 2008, Setter and Lym 2013). Western rockjasmine constituted 10.2%, fringed sage was 7.5%, Drummond false pennyroyal (*Hedeoma drummondii* Benth.) was 3.7%, and tall cinquefoil (*Potentilla arguta* Pursh) was 1.8% of the loamy overflow seedbank in 2004.

The increase in overall percent composition of high seral forbs in 2014 was primarily caused by an increase in rockcress species (*Arabis* spp.), which constituted 9.23% of the seedbank collected in 2014 (Table 5), in addition to western rockjasmine (9.5%) and fringed sage (2.3%). The rockcress species was likely either hairy rockcress [*Arabis hirsuta* (L.) Scop.] or holboelli's rockcress (*Arabis holboelli* Hornem.) which are both native species in the mustard (*Brassicaceae*) family. Hairy rockcress grows well on chalk and limestone slopes, dunes, and dry banks, and prefers well-drained soils (PFAF 2016a) all of which commonly occur in the LMNG. There were 13 new species which appeared in 2014 that were not present any of the previous years, but each constituted < 0.2% of the loamy overflow seedbank (Tables 5 and 6). Conversely, 14 species that were previously recorded in one or more years were absent in 2014 (Table 7). Wild strawberry (*Fragaria virginiana* Duchesn) was observed in 1999 and constituted 0.8% of the seedbank, while tall cinquefoil constituted 2% of the seedbank in 2004 and was also observed in 2009 (Setter and Lym 2013). The remaining absent species had previously constituted < 0.2% of the loamy overflow seedbank each year.

Low seral forb composition increased in the loamy overflow sites from only 297 seedlings in 1999 to 2,809 seedlings in 2014 (Table 4), which constituted approximately 6 and 31% of the seedbank composition, respectively (Setter and Lym 2013) (Table 5). Seven species were observed in the loamy overflow sites every collection date, white sage (*Artemisia*

Table 6. Plant species which emerged in 2014 but were not previously recorded in 12 loamy overflow sites in the Little Missouri National Grasslands in southwestern North Dakota.

Plant category	Species common name	
High seral forbs	Field pussytoes	Suckley's endolepis
	Missouri milkvetch	Rubber rabbitbrush
	Harebell	American water horehound
	Fireweed	Fringed loosestrife
	Flodman's thistle	Tufted evening primrose
	Bastard toadflax	Double twinpod
	Shrubby cinquefoil	
Low seral forbs	Redroot amaranth	Cutleaf evening primrose
	Carolina whitlowort	Prostrate knotweed
	Stickywilly	Littleleaf buttercup
	American licorice	Canada goldenrod
	Prickly lettuce	Field pennycress
	Black medic	Neckweed
High seral grasses	Sideoats grama	Mat muhly
	Buffalograss	Western wheatgrass
	Marsh muhly	Prairie cordgrass
Low seral grasses	Crested wheatgrass	Witchgrass
	Japanese brome	
Hydric/Mesic	Fragrant flatsedge	Needle spikerush

Table 7. Plant species that emerged in previous years but were absent in 2014 in 12 loamy overflow sites in the Little Missouri National Grasslands in southwestern North Dakota.

		Species	
Plant category	Year	Scientific name	Common name
High seral forbs	2009	*Allium textile* A. Nelson & J.F. Macbr.	Wild onion
	2004	*Aster oblongifolis* Nutt.	Aromatic aster
	2009	*Astragalus agrestis* Don	Purple milkvetch
	2004	*Chenopodium gigantospermum* Aellen	Maple-leaved goosefoot
	2009	*Erysimum inconspicuum* (S. Watson) MacMill.	Shy wallflower
	1999	*Fragaria virginiana* Duchesn	Wild strawberry
	2009	*Galium triflorum* Michx.	Fragrant bedstraw
	2004	*Gaura coccinea* var. *glabra* (Lehm.) T. & G.	Scarlet gaura
	2004, 2009	*Gutierrezia sarothrae* (Pursh) Britt. & Rusby	Broom snakeweed

Table 7. Plant species that emerged in previous years but were absent in 2014 in 12 loamy overflow sites in the Little Missouri National Grasslands in southwestern North Dakota (continued).

Plant category	Year	Species: Scientific name	Species: Common name
	1999, 2004	*Hedeoma drummondii* Benth.	Drummond false pennyroyal
	2009	*Oxytropis* spp. DC.	Locoweed
	2004, 2009	*Potentilla arguta* Pursh	Tall cinquefoil
	2009	*Senecio plattensis* (Nutt.) W.A. Weber & A. Löve	Prairie groundsel
	2009	*Solidago rigidum* L.	Stiff goldenrod
Low seral forbs	1999	*Chenopodium rubrum* L.	Red goosefoot
	1999	*Convolvulus arvensis* L.	Field bindweed
	1999, 2004, 2009	*Descurainia pinnata* (Walt.) Britt.	Tansy mustard
	2009	*Erysimum asperum* (Nutt.) DC.	Western wallflower
	1999, 2004	*Erysimum cheiranthoides L.*	Wormseed wallflower
	1999, 2004	*Euphorbia glyptosperma* Engelm.	Ridge-seeded spurge
	2004	*Neslia paniculata* (L.) Desv.	Ball mustard
	2004	*Plantago elongata* Pursh	Slender plantain
	1999	*Plantago major* L.	Common plantain
	1999	*Polygonum convolvulus* L.	Wild buckwheat
	1999	*Rosa arkansana* Porter	Prairie rose
	2004	*Rumex crispus* L.	Curly dock
	1999	*Sonchus* spp.	Sowthistles
	1999	*Verbascum thapsus* L.	Common mullein
High seral grasses	1999	*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffith	Blue grama
	2009	*Hesperostipa spartea* (Trin.) Barkworth	Porcupine grass
	1999	*Muhlenbergia cuspidata* (Torr. ex Hook) Rydb.	Plains muhly
	2004	*Poa palustris* L.	Fowl bluegrass
	2004	*Puccinellia nuttalliana* (Schult.) A. Hitchc.	Nuttal’s alkaligrass
Low seral grasses	2009	*Agrostis scabra* Willd.	Rough bentgrass
	2009	*Bromus tectorum* L.	Cheatgrass
	1999	*Digitaria ischaemum* (Schreb.) Schreb. Ex	Smooth crabgrass
	2009	*Distichlis spicata* (L.) Greene	Saltgrass
	1999	*Echinochola crus-galli* (L.)	Barnyardgrass
	1999, 2004	*Schedonnardus paniculatus* (Nutt.)	Tumblegrass
	1999	*Setaria viridis* (L.) Beauv.	Green foxtail

Table 7. Plant species that emerged in previous years but were absent in 2014 in 12 loamy overflow sites in the Little Missouri National Grasslands in southwestern North Dakota (continued).

		Species	
Plant category	Year	Scientific name	Common name
	2009	*Vulpia octoflora* (Walter) Rydb.	Sixweeks fescue
Unknowns	2009	*Lamiaceae* spp.	Mint family unknown
	2009		Unknown spp. 1
	2009		Unknown spp. 2

ludoviciana Nutt.), Canada thistle, common yarrow (*Achillea millefolium* L.), horseweed [*Conzya canadensis* (L.) Cronq.], greenflower peppergrass (*Lepidium densiflorum* Schrad.), prairie coneflower [*Ratibida columnifera* (Nutt.) Woot. & Standl.], and common dandelion (*Taraxacum officinale* F. H. Wigg). White sage constituted 1% of the seedbank in 1999 with 25 seedlings (Setter and Lym 2013), and increased to 1.4% of the seedbank in 2014 with 130 seedlings (Table 5). Common yarrow constituted approximately 2% of the seedbank consistently from 2004 through 2014; whereas, common dandelion declined steadily from 128 seedlings constituting nearly 3% of the seedbank in 1999 to 44 seedlings and 0.5% of the seedbank in 2014. White sage and common yarrow are both native perennials that commonly occur on plains in a range of soil types (Stubbendieck et al. 2011). Canada thistle is an exotic invasive and noxious weed common to grasslands (Lym and Duncan 2005), but the weed did not constitute more than 0.02% of the seedbank during any year evaluated (Setter and Lym 2013) (Table 5).

The rise in low seral forbs in 2014 can be partially attributed to white sage, flixweed [*Descurainia sophia* (L.) Webb ex Prantl], willowherb (*Epilobium ciliatum* Raf.), rough false pennyroyal (*Hedeoma hispida* Pursh), and Norwegian cinquefoil (*Potentilla norvegica* L.), which together constituted 10.82% of the loamy overflow seedbank in 2014 (Table 5), compared to < 0.2% in 2009 (Setter and Lym 2013). The appearance of new species in the seedbank also factored into the rise of low seral forbs. There were 12 new species recorded in 2014 in the loamy overflow sites (Table 6). The new species constituted approximately 11% of the seedbank, 9% of which was attributed to Carolina whitlowort [*Draba reptans* (Lam.) Fernald]. Carolina whitlowort was the most abundant low seral forb in 2014 with 836 seedlings and is very similar to yellow whitlowort (*Draba nemorosa* L.) which constituted 25% of the loamy overflow seedbank in 2004, and 2.69% in 2014 (Table 5).

There were 14 low seral forb species that were previously recorded in one or more years but absent in 2014 (Table 7). Ball mustard [*Neslia paniculata* (L.) Desv.] constituted 2% of the seedbank in 2004; while, tansy mustard [*Descurainia pinnata* (Walt.) Britt.] was absent in 2014 but was observed every other year, and constituted 3% of the seedbank in 2009 (Setter and Lym 2013). Wormseed wallflower (*Erysimum cheiranthoides* L.) and ridge-seeded spurge (*Euphorbia glyptosperma* Engelm.) were recorded in both 1999 and 2004 and constituted 0.7 and 0.6% of the loamy overflow seedbanks in 2004, respectively. The remaining species which were previously recorded but not found in 2014 constituted < 0.3% of the seedbank each year.

High seral grasses increased across years, with the highest number of seedlings (804) emerging in 2009 (Table 4). The rise from 2004 to 2009 was due to presence of prairie Junegrass [*Koeleria macrantha* (Ledeb.) Schult.] which constituted 13% of the overall seedbank composition with 758 seedlings (Setter and Lym 2013). Prairie Junegrass was still the most abundant high seral grass in 2014 but constituted only 1.9% of the loamy overflow seedbank with 172 seedlings (Table 5); whereas, the most abundant high seral grass in 2004 was green needlegrass, constituting 1.65% of the seedbank (Setter and Lym 2013). Green needlegrass, little bluestem, and sand dropseed [*Sporobolus cryptandrus* (Torr.) A. Gray] were found every year in both ecological sites. Six high seral grasses were absent prior to 2014 and included sideoats grama, buffalograss, marsh muhly [*Muhlenbergia racemosa* (Michx.) Britton, Sterns, & Poggenb.], mat muhly [*Muhlenbergia richardsonis* (Trin.) Rydb.], western wheatgrass, and prairie cordgrass (*Spartina pectinata* Bosc ex Link) (Table 6), all of which constituted a total of less than 0.7% of the loamy overflow seedbank in 2014 (Table 5). There were five high seral grasses absent in 2014 but observed in previous years (Table 7). Fowl bluegrass (*Poa palustris* L.) constituted approximately 1% of the seedbank in 2004, but the remaining four individual species constituted < 0.1% during each year observed (Setter and Lym 2013).

Low seral grasses increased 91% from 42 seedlings (< 1% of the seedbank) in 1999 to 442 seedlings in 2014 (4.9% of the seedbank) (Tables 4 and 5) (Setter and Lym 2013); however, the increase was primarily caused by the introduction of Japanese brome (*Bromus arvensis* L.), which constituted 3.9% of the seedbank in 2014 (Table 5). Japanese brome is an aggressive introduced species which outcompetes desirable vegetation and reduces forage production of perennial grasses as well as grazing performance (Sedivec and Barker 1998). In addition to Japanese brome, witchgrass (*Panicum capillare* L.) was also observed for the first time in 2014 in the loamy overflow sites while crested wheatgrass was found in both ecological sites (Table 6). There were no low seral grasses that were present during all years in either ecological site (Setter and Lym 2013). Eight low seral grasses were observed in previous years but absent in 2014, although each species constituted < 0.4% of the seedbank during the year of observation (Table 7).

Hydric/mesic species were a very small component (< 1%) of the loamy overflow seedbank from 1999 through 2009 with an average of 27 seedlings, but constituted over 9% of the seedbank composition in 2014 with 840 seedlings (Tables 4 and 5). The increase in the number hydric/mesic species was largely due to the presence of needle spikerush [*Eleocharis* *acicularis* (L.) Roem. & Schult.], which constituted 8.6% of the seedbank in 2014 with 784 seedlings and was not found in previous years (Tables 5 and 6). Needle spikerush is a very small sedge with thin hair-like stems which forms mats with dense colonies of plants and prefers to grow in areas with water present such as wet meadows and edges of streams. Similarly, fragrant flatsedge (*Cyperus oderatus* L.), another species which prefers wet and muddy areas, also emerged in 2014 for the first time. The presence of these species in 2014 indicate that the loamy overflow sites had adequate moisture for semi-aquatic plants to establish. Baltic rush (*Juncus* spp.) was the only hydric species recorded every year in the loamy overflow sites (Setter and Lym 2013).

No unknown species were recorded in 1999 or 2004 in either ecological site (Table 4) (Setter and Lym 2013). In 2009, 3 species with a total of 16 seedlings were unable to be positively identified, and 6 species totaling 10 seedlings were unable to be identified in 2014 in the loamy overflow sites (Tables 4 and 5). The unknown species constituted an average of 0.2% of the loamy overflow seedbank across years and contributed very little to seedbank change over time.

Loamy Seedbank

Leafy spurge seedling emergence decreased 87% in the loamy seedbank, from 1,429 seedlings in 1999 to 184 seedlings in 2014 (Table 4), constituting 6% of the seedbank composition 15 yr after *Aphthona* spp. were released (Table 8). Another invasive species, Kentucky bluegrass, constituted 8% of the seedbank in 1999 with 160 seedlings and increased to 1,095 seedlings in 2014 constituting 36.49% of the seedbank (Setter and Lym 2013) (Tables 4 and 8). In 15 yr, Kentucky bluegrass had replaced leafy spurge composition in the seedbank.

Table 8. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999.

			2014	
Scientific name[a]	Common name	C-value[b]	No.[c]	%[d]
Major invasives				
Euphorbia esula L.	Leafy spurge	NV	184	6.12
Poa pratensis L.	Kentucky bluegrass	NV	1,095	36.40
Subtotal			1,279	42.52
High seral forbs				
Androsace occidentalis Pursh	Western rockjasmine	5	68	2.26
Arabis spp.	Rockcress spp.	5-7	228	7.58
Artemisia frigida Willd.	Fringed sage	4	145	4.82
Astragalus missouriensis Nutt.	Missouri milkvetch	7	5	0.17
Campanula rotundifolia L.	Harebell	7	31	1.03
Comandra umbellata (L.) Nutt.	Bastard toadflax	8	28	0.93
Ericameria nauseosa (Pall. ex Pursh) G.L. Nesom & Baird	Rubber rabbitbrush	4	2	0.07
Galium boreale L.	Northern bedstraw	4	7	0.23
Huechera richardsoni R. Br.	Richardson's alumroot	8	1	0.03
Linum perenne Pursh var. *lewisii*	Prarie flax	6	17	0.57
Lithospermum canescens (Michx.) Lehm.	Hoary puccoon	7	4	0.13
Mondarda fistulosa L.	Wild bergamot	5	3	0.10
Oenothera caespitosa Nutt. ssp. *caespitosa*	Tufted evening primrose	8	1	0.03
Physaria brassicoides Rydb.	Double twinpod	8	76	2.53
Subtotal			616	20.48
Low seral forbs				
Achillea millefolium L.	Common yarrow	3	59	1.96
Artemisia ludoviciana Nutt.	White sage	3	3	0.10
Cerastium arvense L.	Prairie chickweed	2	2	0.07
Chamaesyce serpyllifolia (Pers.) Small	Thymeleaf sandmat	0	3	0.10
Chenopodium album L.	Common lambsquarters	NV	1	0.03
Cirsium vulgare (Savi.) Ten.	Bull thistle	NV	1	0.03
Conyza canadensis (L.) Cronq.	Horseweed	0	6	0.20

Table 8. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999 (continued).

Scientific name[a]	Common name	C-value[b]	2014 No.[c]	2014 %[d]
Descurainia sophia (L.) Webb ex Prantl	Flixweed	NV	19	0.63
Draba nemorosa L.	Yellow whitlowort	1	9	0.30
Draba reptans (Lam.) Fernald	Carolina whitlowort	1	233	7.75
Epilobium ciliatum Raf.	Willowherb	3	8	0.27
Erigeron annuus (L.) Pers.	Daisy fleabane	2	34	1.13
Galium aparine L.	Stickywilly	0	2	0.07
Grindelia squarrosa (Pursh) Dun.	Curly-cup gumweed	1	2	0.07
Hedeoma hispida Pursh	Rough false pennyroyal	2	219	7.28
Hyoscyamus niger L.	Black henbane	NV	1	0.03
Lappula occidentalis (S. Watson) Greene var. cupulata (A. Gray) Higgins	Flatspine stickseed	2	2	0.07
Lepidium densiflorum Schrad.	Greenflower peppergrass	0	24	0.80
Medicago lupulina L.	Black medick	NV	1	0.03
Melilotus officinalis (L.) Lam.	Yellow sweetclover	NV	7	0.23
Oenothera laciniata Hill	Cutleaf evening primrose	1	7	0.23
Plantago patagonica Jacq.	Woolly plantain	1	32	1.06
Potentilla norvegica L.	Norwegian cinquefoil	0	48	1.60
Ratibida columnifera (Nutt.) Woot. & Standl.	Prairie coneflower	3	26	0.86
Rumex crispus L.	Curly dock	NV	2	0.07
Salix alba L.	White willow	NV	1	0.03
Silene noctiflora L.	Night-flowering catchfly	NV	11	0.37
Solidago canadensis L.	Canada goldenrod	1	2	0.07
Symphyotrichum ericoides (L.) G.L. Nesom var. *ericoides*	White prairie aster	2	2	0.07
Taraxacum officinale F. H. Wigg	Common dandelion	NV	36	1.20
Thlaspi arvense L.	Field pennycress	NV	8	0.27
Tragopogon dubius Scop.	Yellow salsify	NV	2	0.07
Verbena bracteata Cav. ex lag. & Rodr.	Bigbract verbena	0	2	0.07
Subtotal			815	27.09

Table 8. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999 (continued).

Scientific name[a]	Common name	C-value[b]	2014 No.[c]	2014 %[d]
High seral grasses				
Bouteloua curtipendula (Michx.) Torr.	Sideoats grama	5	2	0.07
Bouteloua dactyloides (Nutt.) J.T. Columbus	Buffalograss	4	4	0.13
Calamovilfa longifolia (Hook) Scribn.	Prairie sandreed	5	35	1.16
Elymus trachycaulus (Link) Gould ex Shinners	Slender wheatgrass	6	3	0.10
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	7	82	2.73
Muhlenbergia richardsonis (Trin.) Rydb.	Mat muhly	10	11	0.37
Nassella viridula (Trin.) Barkworth	Green needlegrass	5	6	0.20
Pascopyrum smithii (Rydb.) Á. Löve	Western wheatgrass	4	3	0.10
Schizachyrium scoparium (Michx.) Nash-Gould	Little bluestem	6	43	1.43
Sporobolus cryptandrus (Torr.) A. Gray	Sand dropseed	6	20	0.66
Subtotal			209	6.95
Low seral grasses				
Agropyron cristatum (L.) Gaertn	Crested wheatgrass	NV	2	0.07
Agrostis scabra Willd.	Rough bentgrass	1	10	0.33
Bromus arvensis L.	Japanese brome	NV	4	0.13
Elymus caninus (L.) L.	Bearded wheatgrass	NV	6	0.20
Elymus repens (L.) Gould	Quackgrass	NV	6	0.20
Poa compressa L.	Canada bluegrass	NV	14	0.47
Subtotal			42	1.40
Hydric species				
Carex spp. L.	Sedges	NV	11	0.37
Cyperus odoeratus L.	Fragrant flatsedge	2	8	0.27
Juncus spp. L.	Rushes	NV	1	0.03
Typha spp. L.	Cattails	NV	15	0.50
Subtotal			35	1.16

Table 8. Scientific name, common name, coefficient of conservatism value, and number of seedlings of plant species that emerged from soil cores excavated in August, 2014 from 12 loamy sites in southwestern North Dakota, 15 yr after *Aphthona* spp. release for leafy spurge control in 1999 (continued).

			2014	
Scientific name[a]	Common name	C-value[b]	No.[c]	%[d]
Unknown species				
Unknown spp. 7 - 9		NV	12	0.40
Subtotal			12	0.40
Total			3,008	100

[a]Scientific nomenclature follows the Flora of the Great Plains (Great Plains Flora Association 1986), except as amended according to the United States Department of Agriculture (USDA)/Natural Resources Conservation Service (NRCS) Plants Database (2006).
[b]Coefficient of Conservatism (C-value) was assigned to plant species based on an assessment by the Northern Great Plains Floristic Quality Assessment Panel (2001). A coefficient value of 'NV' (no value) indicates an introduced or unidentified species; 0-3 indicates species that flourish in highly disturbed habitats; and values of 4-10 indicate species from less disturbed, natural areas.
[c]Total number of seedlings that emerged per 0.5 m^2 from soil samples collected to a depth of 5 cm.
[d]Percentage of total seedlings that emerged across all soil cores.

High seral forbs in the loamy sites increased 85% overall from 89 seedlings in 1999 to 616 seedlings in 2014 (Table 4). Western rockjasmine and fringed sage were observed every year in both ecological sites (Setter and Lym 2013) (Table 8). Across years, western rockjasmine and fringed sage increased steadily in the loamy seedbank, and constituted an average of 1.35 and 5%, respectively, across years. Western rockjasmine reached a maximum of 2.3% in 2014; whereas, fringed sage reached a maximum in 2004 as 10% of the seedbank. Western rockjasmine is a tiny native annual plant in the Primrose (*Primulaceae)* family which is very common in dry sandy soil, open meadows, and disturbed areas (Minnesota Wildflowers 2016a). Therefore, the presence of this species in the seedbank is to be expected because the samples came from an area that was primarily open meadows and had been disturbed by biological control agents and subsequent changes in leafy spurge infestations.

Rockcress species primarily contributed to the increase in high seral forbs and constituted 7.58% in the loamy seedbank in 2014 (Table 8). Both harebell (*Campanula rotundifolia* L.) and hoary puccoon [*Lithospermum canescens* (Michx.) Lehm.] first appeared in 2004, reached a maximum of 5% and 11% in 2009, but declined to 1.03 and 0.13% of the loamy seedbank in 2014, respectively (Setter and Lym 2013). There were 7 new species of high seral forbs observed in 2014 which were absent previous years, and together constituted almost 4% of the seedbank (Table 9). The most abundant new species were bastard toadflax [*Comandra umbellata* (L.) Nutt.] and double twinpod (*Physaria brassicoides* Rydb.) which constituted 0.93 and 2.53% of the loamy seedbank in 2014, respectively. However, 13 species which had been recorded previously were not observed in 2014 (Table 10).

Low seral forb emergence varied throughout the years (Table 4). Low seral forb species constituted 9% of the seedbank in 1999 with 180 seedlings, increased to 47% in 2004 with 1,314 seedlings (Setter and Lym 2013), and declined to 27.09% by 2014 with 815 seedlings (Tables 4 and 8). White prairie aster [*Symphyotrichum ericoides* (L.) G.L. Nesom var. *ericoides*] was recorded every year in the loamy sites in addition to 5 species that were found every year in both ecological sites which included common yarrow, horseweed, greenflower peppergrass, prairie coneflower, and common dandelion.

There were 11 new species recorded in 2014 (Table 9), four of which were found in both ecological sites (Tables 6 and 9) and included Carolina whitlowort, stickywilly (*Galium aparine* L.), cutleaf evening primrose (*Oenothera laciniata* Hill), and field pennycress (*Thalspi arvense* L.). Conversely, 15 species were previously recorded but not observed in 2014 (Table 10). In both ecological sites, western wallflower [*Erysimum asperum* (Nutt.) DC.], ball mustard, slender

Table 9. Plant species which emerged in 2014 but were not previously recorded in the loamy sites in the Little Missouri National Grasslands in southwestern North Dakota.

Plant category	Species common name	
High seral forbs	Missouri milkvetch	Wild bergamot
	Bastard toadflax	Tufted evening primrose
	Rubber rabbitbrush	Double twinpod
	Richardson's alumroot	
Low seral forbs	Prairie chickweed	Curly dock
	Bull thistle	White willow
	Carolina whitlowort	Canada goldenrod
	Stickywilly	Field pennycress
	Black henbane	Bigbract verbena
	Cutleaf evening primrose	
High seral grasses	Sideoats grama	Western wheatgrass
	Buffalograss	Little bluestem
	Slender wheatgrass	
Low seral grasses	Crested wheatgrass	Bearded wheatgrass
	Rough bentgrass	
Hydric / Mesic	Fragrant flatsedge	

Table 10. Plant species that emerged in previous years but were absent in 2014 in the loamy sites in the Little Missouri National Grasslands in southwestern North Dakota.

		Species	
Plant category	Year	Scientific name	Common name
High seral forbs	2004, 2009	*Allium textile* A. Nelson & J.F. Macbr.	Wild onion
	1999, 2004, 2009	*Artemisia campestris* L. ssp. *caudate* (Michx.) Hall & Clem.	Field sagewort
	2009	*Artemisia dracunculus* L.	Silky wormwood
	2004	*Aster oblongifolis* Nutt.	Aromatic aster
	2009	*Erysimum inconspicuum* (S.Watson) MacMill.	Shy wallflower
	1999	*Fragaria virginiana* Duchesn.	Wild strawberry
	2004, 2009	*Gutierrezia sarothrae* (Pursh) Britt. & Rusby	Broom snakeweed
	1999, 2004	*Hedeoma drummondii* Benth.	Drummond false pennyroyal
	2004, 2009	*Lesquerella arenosa* var. *arenosa* (Richardson) Rydb.	Great Plains bladderpod
	2009	*Orthocarpus luteus* Nutt.	Yellow owl's clover
	2004	*Potentilla arguta* Pursh	Tall cinquefoil
	2009	*Senecio plattensis* (Nutt.) W.A. Weber & A. Löve	Prairie groundsel
	2009	*Solidago rigidum* L.	Stiff goldenrod

Table 10. Plant species that emerged in previous years but were absent in 2014 in the loamy sites in the Little Missouri National Grasslands in southwestern North Dakota (continued).

		Species	
Plant category	Year	Scientific name	Common name
	1999	*Solidago* spp.	Goldenrods
Low seral forbs	2009	*Cirsium arvense* (L.)	Canada thistle
	1999, 2004	*Descurainia pinnata* (Walt.) Britt.	Tansy mustard
	1999, 2009	*Erysimum asperum* (Nutt.) DC.	Western wallflower
	1999, 2004	*Erysimum cheiranthoides L.*	Wormseed wallflower
	1999, 2004	*Euphorbia glyptosperma* Engelm.	Ridge-seeded spurge
	2004	*Euphorbia prostrata* Ait.	Prostrate spurge
	1999	*Lactuca serriola* L.	Prickly lettuce
	2009	*Medicago sativa* L.	Alfalfa
	2004	*Neslia paniculata* (L.) Desv.	Ball mustard
	2004	*Oenothera biennis* L.	Common evening primrose
	2004	*Plantago elongata* Pursh	Slender plantain
	1999	*Plantago major* L.	Common plantain
	1999	*Sisymbrium altissimum* L.	Tumble mustard
	1999	*Sonchus* spp.	Sowthistles
	1999	*Verbascum thapsus* L.	Common mullein
High seral grasses	2004	*Agropyron spicatum* (Pursh) Scribn. & Sm.	Beardless wheatgrass
	1999	*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex	Blue grama
	1999, 2009	*Hesperostipa comata* (Trin. & Rupr.) Barkworth	Needle-and-thread
	2004, 2009	*Hesperostipa spartea* Trin.	Porcupine grass
	1999	*Muhlenbergia cuspidata* (Torr. Ex Hook) Rydb.	Plains muhly
	2004	*Poa palustris* L.	Fowl bluegrass
	2004	*Puccinellia nuttalliana* (Schult.) A. Hitchc.	Nuttal's alkaligrass
	2009	*Sporobolus compositus* (Poir.) Merr.	Composite dropseed
Low seral grasses	2004	*Bromus inermis* ssp. *Inermis*	Smooth brome
	1999	*Echinochola crus-galli* (L.)	Barnyardgrass
	1999, 2004	*Schedonnardus paniculatus* (Nutt.)	Tumblegrass
	2009	*Lamiaceae* spp.	Mint family unknown
	2009		Unknown spp. 1
	2009		Unknown spp. 2
	2009		Unknown spp. 3

plantain (*Plantago elongata* Pursh), common plantain (*Plantago major* L.), common mullein (*Verbascum thapsus* L.) were absent in 2014 (Tables 7 and 10).

Common yarrow increased steadily from 0.10% in 1999 to almost 2% in 2014 (Setter and Lym 2013) (Table 8). The amount of horseweed varied throughout the years, and began with 1% in 1999, reached a maximum of 2% in 2004, and declined to 0.2% in 2014. Dandelion constituted 2% of the seedbank in 1999, reached a maximum of just over 4% in 2004, and decreased to 1.2% of the seedbank in 2014. Rough false pennyroyal constituted 0.3% of the seedbank and was first observed in 2009 but increased to 7.28% by 2014. Common mullein was the most abundant species in 1999 constituting 4% of the seedbank, but was absent thereafter. Likewise, wormseed wallflower and ball mustard constituted approximately 5% each in 2004, and were not observed again. Similar to the loamy overflow sites, yellow whitlowort constituted almost 30% of the loamy seedbank in 2004, 22% in 2009, and was the most abundant species both years, but decreased to 0.3% in 2014. Carolina whitlowort which was not previously recorded constituted 7.8% of the seedbank in 2014 as the most abundant low seral forb. Carolina whitlowort is a very small plant which grows abundantly especially in areas with heavy grazing, indicating that overgrazing may have likely contributed to the presence and abundance of Carolina whitlowort in the seedbank (Minnesota Wildflowers 2016b). Also, common yarrow, dandelion, and mullein frequently grow in areas with disturbed soils (Stubbendieck et al. 2011). So, a decrease in these low seral species may indicate that the quality of the sites has improved throughout the years.

High seral grass composition has not changed for the last 15 yr (Table 4), 168 seedlings constituted 8% of the seedbank in 1999 and 209 seedlings constituted 7% of the seedbank in 2014 (Setter and Lym 2013) (Tables 4 and 8). Prairie Junegrass was the most abundant species in 1999, 2009, and 2014, and constituted approximately 4, 14, and 3% of the loamy seedbanks, respectively (Setter and Lym 2013) (Table 8). Prairie sandreed was found every year in the loamy sites, in addition to green needlegrass, little bluestem, and sand dropseed which were found every year in both ecological sites. Prairie Junegrass, prairie sandreed, green needlegrass, little bluestem, and sand dropseed are native perennial grasses common to prairies and open areas (Stubbendieck et al. 2011). Prairie sandreed composition varied throughout the years, constituting 2% of the loamy seedbank in 1999, reaching a maximum of 4.2% in 2004, declining to about 1.2% in 2014.

There were three new high seral grass species observed in 2014 which were common to both ecological sites and included sideoats grama, buffalograss, and western wheatgrass (Tables 6 and 9), while slender wheatgrass [*Elymus trachycaulus* (Link) Gould ex Shinners] was a new species in 2014 only in the loamy sites (Table 9). Sideoats grama and buffalograss are species which prefer dry well, drained soils on hills and open prairies (Stubbendieck et al. 2011). Species previously recorded in both ecological sites yet absent in 2014 included fowl bluegrass, Nuttall's alkaligrass [*Puccinellia nuttalliana* (Schult.) A. Hitchc.], blue grama, Plains muhly [*Muhlenbergia cuspidata* (Torr. ex Hook) Rydb.], and porcupinegrass (*Hesperostipa spartea* Trin.), while composite dropseed [*Sporobolus compositus* (Poir.) Merr.], bluebunch wheatgrass [*Pseudoroegneria spicatum* (Pursh) Á. Löve], and needle-and-thread [*Hesperostipa comata* (Trin. & Rupr.) Barkworth] were previously recorded only in the loamy sites (Setter and Lym 2013) (Tables 7 and 10).

Low seral grass emergence in the loamy sites increased from 6 seedlings in 1999 to 42 seedlings in 2014 (Table 4). The overall increase in low seral grasses in the loamy sites was due to an increase in Canada bluegrass (*Poa compressa* L.) which was first recorded in 2009 with just 1 seedling, but increased to 14 seedlings in 2014 (Table 8). The invasive species, Japanese brome, only constituted 0.13% of the loamy seedbank. Rough bentgrass (*Agrostis scabra*

Willd.) and bearded wheatgrass [*Elymus caninus* (L.) L.] were found only in the loamy sites in 2014 (Table 9), while crested wheatgrass [*Agropyron cristatum* (L.) Gaertn] was in both ecological sites (Tables 6 and 9). Crested wheatgrass is an introduced species which has become naturalized and is commonly included in re-seeding mixtures (McArthur 1988). Species absent in 2014 but recorded in previous years included smooth brome (*Bromus inermis* ssp. *inermis)* and tumblegrass [*Schedonnardus paniculatus* (Nutt.)] in the loamy sites (Table 10) and barnyardgrass [*Echinochola crus-galli* (L.)] in both ecological sites (Tables 7 and 10).

Hydric/mesic species emergence in the loamy sites increased slightly from 20 seedlings in 1999 to 35 seedlings in 2014 (Table 4). In 1999, sedge species were the most abundant hydric species constituting 0.9% of the seedbank, but cattails (*Typha* spp.) were the most abundant from 2004 through 2014 (Setter and Lym 2013) (Table 8). All hydric/mesic species that were previously recorded in the loamy sites were recorded again in 2014 (Setter and Lym 2013) (Table 8). Additionally, cattails and various sedges were recorded every year in both ecological sites. Fragrant flatsedge emerged for the first time in 2014 in both ecological sites (Tables 6 and 9).

There were 13 and 12 unknown seedlings in the loamy sites in 2009 and 2014, respectively (Table 4). Averaging across years, the unknown species constituted 1% of the loamy seedbank; thus, changes in unknown species composition had little affect on the overall changes in seedbank composition (Setter and Lym 2013) (Table 8).

Summary

As leafy spurge was controlled, Kentucky bluegrass began to infest both ecological sites. The decrease in cover and seed production reduced competition from leafy spurge and allowed Kentucky bluegrass the opportunity to invade and become the more dominant species.

Continued livestock grazing during years of optimal precipitation has also likely contributed to the rapid increase and invasion of Kentucky bluegrass (Patton et al. 2007). Yet, grazing presence and reduced competition likely affected the rate of increase more than precipitation alone which has been variable throughout the last 15 yr and has averaged 320 mm since 1999 (NDAWN 2016, averaged between Beach and Dickinson, ND). However, the rate of spread and production of Kentucky bluegrass, especially in the loamy overflow sites, has been deterred by the slow shift and reintroduction of native species into the seedbank through the last 5 yr. Species richness doubled in both ecological sites since 1999. Often, plant communities which contain more species are more productive; however, the effects of the deletion of an individual species on total biomass is dependent on which species are present in the community and which species are lost.

These results contrast the study of Lesica and Hanna (2004) in which the introduction of biocontrol agents in Montana grasslands was not associated with a disproportionate increase in nontarget exotic species and they speculated that the reduction of weed dominance may not always be associated with increased species diversity. However, the results are very similar to a 14 yr study in Montana in which native diversity increased after the release of *Aphthona* spp. flea beetles and subsequent reduction in leafy spurge (Lesica and Hanna 2009). Moreover, relative to the decline in leafy spurge abundance, the increase in native diversity was small and Lesica and Hanna suggested that increases of native alpha diversity in semiarid grasslands may require decades and that response to a reduction in weed species may depend on site history and conditions.

The introduction of biological control agents drastically reduced leafy spurge infestations and made resources more available for enhanced growth of other individuals already within the community. The introduction of new individuals, many of which likely differ in resource capturing abilities, may alter resource availability patterns in the environment (Bazzaz 1996). Disturbance also alters the acquisition and allocation of resources in plants which interact with each other through modifications of physical and chemical factors in the above- and below-ground environment. Therefore differences in seedbank evaluations could be caused just by disturbances and changes within the plant community. For example, the microenvironment of the seedbed can be drastically altered by the accumulation of herbaceous litter or lack thereof (Young 1988). Litter accumulates seeds by replacing the smooth soil surface with a porous and easily penetrated layer. Also, litter presence changes the range of temperatures and the humidity of the surface below which could affect germination and mortality rates. In contrast, the accumulation of larger plant matter and leaves may also form an overlapping canopy that deflects seeds. Although not present in the seedbank, some sites had dense layers of creeping juniper, which may have prevented more seeds from entering the seedpool, so sites with creeping juniper stands rather than Kentucky bluegrass stands tended to have lower seed emergence.

Plant reproduction varies greatly in the timing and frequency of cycles, the number, size, and quality of the offspring, and the amount of resources allocated towards reproduction (Bazzaz 1996, Harper 1977). The response speed in which allocation patterns react to a change in the availability of resources is closely linked to availability in the environment as well; therefore, early successional environments will be more likely to support species that can quickly shift their resource allocations. The disappearance of plants that were found in previous evaluations but were not observed in 2014 could be a result of a shift in the plant community from early successional plants which are more efficient at colonizing areas and capturing resources to late successional plants which may grow slower and have a better ability to survive longer (Bazzaz 1996).

The change in plant species could also be caused by changes in grazing. Cattle avoid grazing leafy spurge-infested sites leading to intense, frequent use of non-infested sites and unequal grazing distribution, especially early in the grazing season (Lym and Kirby 1987). As a result herbage of preferred species is decreased, herbage production diversity is decreased, and the community is more susceptible to invasion. The reduction of leafy spurge infestations could have increased or altered grazing tendencies in the area, which may have allowed different species to establish and reproduce.

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