Combining Herbicides and Fertilizers to Enhance Control of Leafy Spurge (*Euphorbia virgata* Wald & Kit)

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ABSTRACT

Across the Northern Great Plains, leafy spurge [*Euphorbia virgata* Wald & Kit (previously known as *Euphorbia esula* L.)], is a problematic invasive plant that contributes to the degradation of native ecosystems through displacement of indigenous vegetation. Controlling leafy spurge is extremely challenging, often requiring multiple herbicide applications for sustained suppression. Below ground, leafy spurge associates strongly with arbuscular mycorrhizal fungi (AMF) in a symbiotic plant-soil relationship that typically benefits both the host plant and the fungi by facilitating nutrient and resource exchange. However, in a nutrient rich environment, mycorrhizal associations can be altered such that fertilizers diminish the advantages of AMF, causing the relationship to become harmful to the host plant through depletion of essential fats and sugars. Consequently, the strategic application of fertilizer may be a tool in enhancing control of leafy spurge, effectively transforming the mycorrhizal association from beneficial to parasitic. When combined with herbicide treatments, this approach holds promise for addressing leafy spurge invasion more effectively and sustainably.

Research plots were established in leafy spurge invaded native prairie across four locations in Saskatchewan. Fertilizer and herbicide treatments were applied in June 2022, with fertilizer reapplied in 2023. Herbicide treatments significantly reduced leafy spurge biomass and cover in year one, while in year two only Tordon and Navius significantly reduced leafy spurge. However, these herbicides also decreased species richness through native forb loss resulting in a shift in the plant community toward grass dominance. Select fertilizer treatments, specifically micronutrient and nitrogen, decreased leafy spurge cover but only at the plot level suggesting scale dependent effects. Herbicide and fertilizer effects on AMF were complex, where certain fertilizers, particularly micronutrient and phosphorus, decreased AMF colonization, but only when applied with select herbicides – 2,4-D combined with micronutrients and Tordon combined with phosphorus – suggesting that it is possible to manage AMF through use of fertilizers. Herbicide and fertilizer effects on forage quality were similarly dependent on specific treatment combinations, with crude protein and fiber content all responding to some combination of treatment. Given the complexity of these effects, more research is needed before recommendations can be made to producers.

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DEDICATION

Lovingly dedicated to my husband Jamie for his unwavering love and support.

Thank you for enabling me to live my dreams.

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2,4-D - 2,4-D ester
a.e. - acid equivalent
ADF - acid detergent fiber
ae/ha - acid equivalent per hectare
Al - aluminum
ALS - acetolactate synthase
AMCP - aminocyclopyrachlor
AMF - arbuscular mycorrhizal fungi
ATV - all-terrain vehicle
B - boron
Ca- calcium
Cd - cadmium
CEC - cation exchange capacity
cm - centimeter
cm^2 - centimeter squared
CP - crude protein
Cu - copper
Df - diflufenzopyr
Di - dicamba
Fe - iron
g/ha - gram per hectare
HCl - hydrochloric acid
IF - invasive forbs (not including leafy spurge)
IG - invasive grasses
K - potassium
kg - kilogram
kg/ha - kilogram per hectare
KOH - potassium hydroxide
L/ha - litre per hectare
lbs/ac - pounds per acre
m - meter
m^2 - meters squared
Mg - magnesium
micro - micronutrient
ml - mililitre
Mn - manganese
MSM - metsulfuron-methyl
NDF - neutral detergent fiber
NF - native forbs
NG - native grasses
NH_4 - ammonium
NMDS - non-metric multidimensional scaling
NO_3 - nitrate
NS - native shrubs
P - phosphorus
Pb - lead
PERMANOVA - permutational multivariate analysis of variance
PRS® - plant-root simulator
RM - rural municipality
S - sulfur
SOM - soil organic matter
v/v - volume per volume
Zn - zinc

Chapter 1: Introduction

Invasive plants pose one of the greatest threats to the integrity of North American grasslands (Gaskin et al., 2020). They reduce native plant diversity and alter the surrounding soils through nutrient cycle changes and shifts to the soil microbial community structure and function (Gibbons et al., 2017), resulting in decreased land productivity through decreased forage quality and yield (DiTomaso, 2000). One of the most invasive and problematic weeds is leafy spurge [*Euphorbia virgata* Wald & Kit (previously known as *Euphorbia esula* L.) see Flora of North America (Riina et al., 2020)], which has impacted millions of hectares of grasslands throughout North America (Duncan et al., 2004). Leafy spurge is known to displace native plant species (Butler & Cogan, 2004), alter soil nutrient cycling (Gibbons et al., 2017), lower land productivity (Clay & Scholes, 1997), poison livestock (Messersmith et al., 1985), and alter livestock grazing patterns (Hein & Miller, 1992).

Achieving long term control of leafy spurge is extremely difficult. It reproduces by seed as well as a spreading root system which contributes to its “weediness”(Clay & Scholes, 1997). Additionally, all parts of the plant contain a milky latex that is toxic to most livestock and is therefore avoided during grazing allowing the leafy spurge to increase (Gucker, 2010; Lacey & Sheley, 1996). Selective herbicides are often considered the most effective method of reducing leafy spurge density in grasslands, however broadleaf herbicides can negatively affect native forb and shrub species and shift the plant community toward grasses (Crone et al., 2009). The use of a single control method is often not sustainable long-term (DiTomaso et al., 2010), including repeated use of a single herbicide, which can select for resistance in the targeted weed species and cause soil nutrient changes that alters the health of the grassland ecosystem (DiTomaso, 2000). The number of new herbicides registered for use has dramatically decreased since the 1960’s and 70’s and herbicides labelled for use in grasslands are even further limited (Holm & Johnson, 2009), highlighting the importance of herbicide stewardship (Gaines et al., 2020) along with applying integrated weed management.

Fertilizers are a tool that can be used to increase desired plant competition and thereby suppress invasive species (Cole et al., 2007). Integrating the effects of fertilizers with herbicides can be beneficial. While the herbicide works to control the invasive plant the fertilizer increases the native plant competition (Cole et al., 2007). Thus, the integration of fertilizers with herbicides compliment each other and can improve long-term control over use of either technique alone (Cole et al., 2007).

This research project explores new methods of controlling leafy spurge by combining herbicides and fertilizers. Leafy spurge is strongly associated with arbuscular mycorrhizal fungi (AMF). Normally this is a beneficial relationship for both the host plant and the fungi with the host plant providing sugars and fats in exchange for improved acquisition of soil nutrients (Johnson et al., 2015). However, in a nutrient-rich environment, the symbiotic AMF relationship can switch to parasitic as the cost to supply carbon resources by the host plant outweighs the benefits of the nutrient acquisition from the AMF (Johnson et al., 2015). A long-term study completed by Wu et al. (2022) found that phosphorus (P) and zinc (Zn) were responsible for a reduction in soil fungal diversity and soil community composition. Thus, regulating the levels of P and Zn fertilization may be a way to control soil fungal communities (Wu et al., 2022), and potentially a way to indirectly regulate the growth of AMF-associated plants such as leafy spurge. Additional nutrient applications of P and micronutrients, specifically Zn, may be a way to control soil fungal communities and improve control of leafy spurge, especially when combined with the use of herbicide. Integrating herbicides and fertilizers could reduce the reliance on one single control technique and contribute to sustainability over the long term. However, the combined effects of herbicides and fertilizers on rangeland soils may cause unintended consequences, which could affect the sustainability of this approach and should be quantified.

1.1 Project Objectives

1. Determine an optimal combination of herbicide and fertilizer that maximizes control of leafy spurge and minimizes impacts on native plants.
2. Determine the herbicide and fertilizer combinations that increase grass productivity while effectively controlling leafy spurge.

1.2 Hypotheses

1. Phosphorus (P) and micronutrient fertilizers, particularly zinc (Zn), will reduce leafy spurge abundance by creating a nutrient rich environment and switching the AMF relationship from beneficial to parasitic, especially when combined with herbicide application. As evidence of the change in the AMF relationship, the P and Zn fertilizers will alter AMF colonization.

2. The plant community will be altered as a result of the herbicide applications including reduced plant species richness, and cover of leafy spurge, native forbs, and native shrubs. Non-residual herbicides will have less of an impact on the plant community when compared to residual herbicides.
3. Nitrogen (N), phosphorus (P), and micronutrient fertilizer will increase grass productivity while effectively controlling leafy spurge. Forage quality will also be improved as a result of the fertilizer treatments.

Chapter 2: Literature Review

2.1 Invasive Plant Species

Invasive plant species are plants that are introduced beyond their native range and are able to grow and expand as a self-sustaining population causing negative impacts to the local species and ecosystems (Hess et al., 2019). Invasive plants are known to displace native vegetation, reduce native biodiversity, and alter ecosystem functions (Florianová et al., 2019; Liu et al., 2023). Many invasive plant species form dense monocultures changing the plant community structure and altering primary productivity and nutrient cycling (CFIA, 2008). These changes to the nutrient cycling process subsequently influence the soil microbial community, including arbuscular mycorrhizal fungi (AMF) (Ehrenfeld, 2003). Significant economic loss can also result as a consequence of invasive plants (Callaway et al., 2004; Florianová et al., 2019), including reduced land values, reduced forage production, and increased costs for control (Colautti et al., 2006). Ongoing globalization and the intensified movement of people and goods will continue the introduction of non-native plant species into new habitats, and the potential for these species to become invasive (Kuebbing & Nuñez, 2015; Oschrin & Reynolds, 2019).

Characteristics such as enhanced germination rates, earlier emergence, rapid growth, and the ability to modify soil conditions all provide invasive plant species with a competitive advantage over native species (Hess et al., 2019). Early emergence combined with rapid growth produces a larger plant, benefiting the invasive plant over smaller or later-growing species (Hess et al., 2019). Plus, invasive plants can acquire light, space, water, and nutrients more efficiently than native species leading to increased productivity (Akin-Fajiye et al., 2021; Hess et al., 2019). Many invasive plants produce and exude secondary compounds that can alter the soil microbe community resulting in altered decomposition, nitrogen mineralization and nitrification, and soil enzyme activity (Kalisz et al., 2021; Torres et al., 2021). These changed soil conditions subsequently affect the recruitment and growth of future plants (Forero et al., 2019), a process known as plant-soil feedback. Self-induced soil modification can include biological, chemical, and/or physical changes (Gibbons et al., 2017; Hess et al., 2019; Thorpe & Callaway, 2006). These self-induced soil changes reward the invasive species as it creates a habitat suitable for its own growth (Hess et al., 2019). Invader-induced soil changes may persist even after the species is removed leaving behind a “soil legacy” effect, especially if the invasive plant was present for a long time or was very abundant (Hess et al., 2019).

2.1.1 Leafy Spurge

Introduced from Eurasia (CABI, 2022; Gucker, 2010), leafy spurge [*Euphorbia virgata* Wald & Kit (previously known as *Euphorbia esula* L.) see Flora of North America (Riina et al., 2020)], is an aggressively invasive forb located throughout much of North America including across Canada with a strong prevalence in the prairie provinces (Best et al., 1980). Introduced in Saskatchewan in 1928 (Best et al., 1980), leafy spurge is found in every habitat in the province except the boreal forest (Gucker, 2010). It can be found in dry, subhumid, and even subarctic environments (Best et al., 1980). Leafy spurge prefers coarse-textured, sandy soils with no preference for associating with any particular plant group (Best et al., 1980; Messersmith et al., 1985).

Because of its vigorous and strong competitive character (Messersmith et al., 1985), leafy spurge is listed as one of the worst noxious weeds in Canada and the United States (Gucker, 2010). It infests prime grazing pasture resulting in increased invasive grass presence, diminished native grass productivity, and reduced carrying capacity (Beck, 2013; CABI, 2022; Clay & Scholes, 1997; Liu et al., 2023). In grazed pastures, Liu et al. (2023) found that native forbs became excluded as leafy spurge became more abundant. Shorter and less shade-tolerant plants were excluded as leafy spurge dominated over these for light (Liu et al., 2023). Liu et al. (2023) also found leafy spurge was negatively associated with plant species richness at several study sites across Saskatchewan and grass forage production was reduced as leafy spurge abundance increased (Liu et al., 2023). Thus, leafy spurge can outcompete native vegetation for light, water, nutrients, and space, resulting in reduced species diversity and reduced forage productivity leading to functional loss within the ecosystem (CABI, 2022; Liu et al., 2023).

Leafy spurge emerges in early spring with seedlings growing rapidly, giving it a competitive advantage over native plants (CABI, 2022; Lym, 1998; Messersmith et al., 1985). Emergence in Saskatchewan begins in mid-April (Best et al., 1980; Messersmith et al., 1985) where it grows up to one meter tall from a woody base and develops linear-shaped leaves that appear blue-green in color (Lym, 1998). It produces showy yellow-green bracts borne on umbels that appear in late May, with flowers emerging in mid-June (Lym, 1998).

As a clonal species, leafy spurge has an extensive root system that is most abundant in the upper 12 cm of the soil (Lym, 1998). Vertical roots are typically 2.4 m deep but may reach depths of up to 9 m (Best et al., 1980). Vegetative reproduction via its lateral roots is important for its horizontal spread and is the main contributor to its weediness (Best et al., 1980; Messersmith et al., 1985). A single leafy spurge plant can spread up to 5 m in diameter per year, with small patches expanding at rates up to 500 times faster than large patches, highlighting the need to control small patches promptly (Lym, 1998). Further, leafy spurge roots are protected by a layer of cork which gives them a woody texture and protects them from moisture loss during drought conditions (Clay & Scholes, 1997; Messersmith et al., 1985). Consequently, leafy spurge's deep, tough, and rapidly spreading roots make it a difficult species to eradicate (Best et al., 1980).

In addition to vegetative reproduction, leafy spurge also reproduces by seed, with peak seed production occurring in late spring (Lym, 1998). Leafy spurge is a prolific seed producer with an average seed rain of 2500 seeds/m^2 (Best et al., 1980; Gucker, 2010). Seeds undergo several colour changes as they mature, turning from yellow to orange, to brown, and finally grey at maturity (CABI, 2022). Leafy spurge control should be completed before the seeds turn brown to prevent viable seed production (Lym, 1998). Seed dispersal occurs when the capsule reaches maturity and ruptures ejecting seeds up to 5 m away from the parent plant (Best et al., 1980; Gucker, 2010).

All parts of the leafy spurge plant contain a milky white latex (Best et al., 1980), which deters grazing (Lym, 1998), and may inhibit herbicide translocation (Messersmith et al., 1985). The sticky latex serves as both a physical and chemical defense mechanism to resist herbivory from both insects and grazing mammals (CABI, 2022; Clay & Scholes, 1997), and may even function as an insecticide (Clay & Scholes, 1997). The latex contains a highly irritating and inflammatory compound called ingenol which causes vomiting and diarrhea when ingested, thus making the plant unpalatable to cattle (Lym, 1998).

A common invasive plant mechanism is allelopathy, which is the production of chemicals by a plant that impacts soil microbes or the recruitment of neighbouring plants (Kalisz et al., 2021). Leafy spurge has been shown to inhibit the growth of tomato seedlings, suggesting it may produce and release allelopathic secondary compounds into the soil, impacting both neighbouring plant performance (Qin et al., 2006) and possibly altering soil microbes (Kalisz et al., 2021). Additionally, allelopathic compounds can alter soil nutrient availability for the benefit of the invasive plant species (Kalisz et al., 2021), although it is unknown whether this is the case with leafy spurge.

Leafy spurge associates strongly with AMF, which are considered fundamental for soil nutrient resource acquisition (Kalisz et al., 2021). Lekberg et al. (2013) found that leafy spurge invasions support both higher abundance and diversity of AMF when compared to the uninvaded native plant community. AMF aid in providing the host plant with soil nutrients in exchange for host sugars (Bhantana et al., 2021). The uptake of important macronutrients such as nitrogen (N), phosphorus (P), potassium (K), and sulfur (S), along with micronutrients such as calcium (Ca) and zinc (Zn), is facilitated by AMF (Bhantana et al., 2021). In addition to AMF playing a key role in increasing nutrient use efficiency, AMF also increases the plant’s tolerance to environmental stress such as drought or salinity (Bhantana et al., 2021). The AMF-enhanced uptake of P, copper (Cu), and Zn are associated with helping plants alleviate abiotic stresses (Bhantana et al., 2021). However, when N is limited, AMF are unlikely to release the nutrient to their host until their own needs have been met (Johnson et al., 2015). Thus, plants, AMF, and other soil microbes all compete for N, especially in soils with low N availability (Johnson et al., 2015). Moreover, AMF can become parasitic when the costs to the host overcome the benefits (Lekberg et al., 2021). Johnson et al. (2015) demonstrated that in P-limited soils AMF mutualisms are expected, but in P-rich soils, the AMF association reverses to parasitic. Thus, the use of fertilizer may be an effective tool in controlling leafy spurge by overturning the AMF association from beneficial to parasitic.

2.2 Invasive Plant Species and Soil Nutrients

Invasive plant species can change the species composition of the plant community leading to altered timing and rate of nutrient cycling and availability (Bell et al., 2020). Nutrient availability in ecosystems is a balance between input and loss rates (Cleland & Harpole, 2010). As plants grow and deplete soil nutrients, competition for nutrients increases (Cleland & Harpole, 2010). Increasing the supply of nutrients through fertilization causes plant competition to shift to a new limiting resource resulting in changes in species composition and diversity as different species have an affinity for different resources (Cleland & Harpole, 2010). Nitrogen fertilization experiments have consistently shown that species richness is reduced, and plant community structure is altered, while primary productivity increases (Akin-Fajiye et al., 2021; Dindová et al., 2019; Leff et al., 2015).

Research has shown that nutrient addition in grasslands results in a loss of plant diversity and a shift in the composition of the plant community to faster-growing plants (Leff et al., 2015). These faster-growing plants are better competitors for light when nutrient supply is not limited (Leff et al., 2015). Additionally, soil microbial communities, including AMF, are sensitive to nutrient addition, with Leff et al. (2015) reporting decreased relative abundance of AMF with nutrient addition across several globally distributed grasslands. van der Heijden (2010) found that AMF contribute to ecosystem sustainability by reducing P leaching, especially in nutrient-poor, sandy grassland soils. However, when these soils were fertilized there was a reduced ability by the AMF to regulate the loss of P leaching likely by disrupting the symbiotic relationship between host plants and the AMF in the nutrient-rich environment (van der Heijden, 2010). A long-term study completed by Wu et al. (2022), found that P and Zn were responsible for reduced soil fungal diversity and soil community composition. Thus, regulating the levels of P and Zn fertilization may be a way to control soil fungal communities (Wu et al., 2022), and potentially a way to indirectly regulate the growth of AMF-associated plants, especially invasive plants that associate strongly with AMF such as leafy spurge.

N and P are both important nutrients for plant growth. A study completed by Akin-Fajiye et al. (2021) examined the role of abiotic and biotic factors in driving invasive plant richness in a semi-arid grassland. They tested the relationship of invasive and native plant richness with soil nutrients N, P, and K, and found that invasive plant species richness increased with enhanced N and P in the soil while native plants did not respond (Akin-Fajiye et al., 2021). High soil N availability contributed to reduced native species richness, with an increased invasive species richness, resulting in an alteration to the overall composition of the plant community. Invasive forbs were especially responsive to P with increased P levels increasing their richness, even at low N levels, suggesting invasive plants may have an increased P utilization efficiency compared to native plants (Akin-Fajiye et al., 2021). Thus, enriched soil nutrient availability may enable invasive plants to establish in new environments.

2.2.1 Nitrogen (N)

Nitrogen (N) is a building block of amino acid and protein synthesis and thus is an essential element for all forms of life (Cleland & Harpole, 2010). Although N is known to increase forage production, fertilization of natural grasslands often has several negative outcomes including loss of species richness and community structure (Cleland & Harpole, 2010). Plant community structure is influenced by a plant's ability to compete for soil N and often depends on whether the plant is an early or late successional species (Cathcart et al., 2004). Early successional species, including many invasive plant species, grow more rapidly and acquire more N than late successional species (Cathcart et al., 2004). Additionally, N addition in grasslands has been shown to impact microbial groups responsible for soil N cycling processes, resulting in changes to the soil N cycle and promoting N loss and leaching (Frey et al., 2023). Gill et al. (2022) found the addition of N greatly accelerated early-stage decomposition, while inhibiting later-stage decomposition. Keller et al. (2023) found N addition increased grassland root biomass and turnover. Ren et al. (2019) found that N addition shifted invasive plant biomass allocation to produce greater root biomass increasing its below-ground competitive ability.

2.2.2 Phosphorus (P)

Phosphorus (P) is an essential plant macronutrient that is critical for metabolic processes including plant energy transfer, photosynthesis, and gene replication and expression (Bhantana et al., 2021). AMF symbiosis plays an important role in P acquisition for the host plant, especially with soil-immobile nutrients like P (Bhantana et al., 2021). Recent studies have documented increased P accumulation in Canadian soils, particularly in areas with a high density of livestock (Akin-Fajiye et al., 2021). However, P has been shown to cause native species loss and reduced species diversity (Akin-Fajiye et al., 2021). High levels of soil P also reduce AMF colonization and decrease the abundance and richness of AMF communities (Bhantana et al., 2021). Lekberg et al. (2021) observed that P suppresses AMF but only when P was added together with N, while independent additions of N and P had no effect. Keller et al. (2023) noticed P additions increased grassland root production by 15%. Thus, P nutrient addition can stimulate the growth of invasive plant species and alter competitive outcomes with native plant competitors (Cui et al., 2023).

2.2.3 Micronutrients

Micronutrient fertilizer is designed to increase the efficiency of major nutrients and maximize plant growth by boosting micronutrient levels in the root zone. Micronutrients such as copper (Cu), iron (Fe), and zinc (Zn) play an important role in plant growth and development (Chen et al., 2024). A meta-analysis by Radujković et al. (2021) explored the effects of fertilization experiments on biomass production in grasslands and found that two micronutrients, Zn and Fe, were significantly associated with variation in biomass. Zn, for example, has several beneficial functions for plant health including metabolizing proteins, carbohydrates, and lipids (Bhantana et al., 2021). However, the water solubility of Zn fertilizers affects its availability for plants, as does the pH of the soil (Watts-Williams et al., 2014). Specifically an increase in soil pH decreases the availability of Zn for plant uptake (Watts-Williams et al., 2014). Zn acquisition can be significantly improved for plants through an association with AMF, especially in Zn deficient soils (Watts-Williams et al., 2014). When Zn levels are low, plants experience reduced growth; however, an AMF association can enhance plant P and Zn uptake, especially when soil is deficient in these nutrients (Bhantana et al., 2021). However, the simultaneous application of Zn with P fertilizer can have negative effects on Zn uptake for plants due to complex Zn-P interactions that alter both plant and soil factors (Watts-Williams et al., 2014). Plants associated with AMF had higher P contents than plants with no AMF associations, though this was not the case with Zn, potentially as a result of the P fertilizer added simultaneously (Watts-Williams et al., 2014). Colonization of the mycorrhizal plant was significantly decreased with P addition, regardless of the Zn addition treatment, suggesting inhibition of mutualism functioning (Watts-Williams et al., 2013).

When micronutrient treatments are added in agricultural lands, invasive species commonly outcompete native plants suggesting that micronutrients may be a driver of invasion (Chen et al., 2024). Conversely, in urban soils, micronutrient additions reduced the competitive ability of the invasive species (Chen et al., 2024). Consequently, micronutrients have the potential to enhance or reduce invasion, but it is unclear what effects they will have in grasslands.

2.3 Leafy Spurge and Soil Nutrients

Leafy spurge is sensitive to N deficiency as demonstrated by the significant reduction in shoot height, growth of auxiliary buds, and shoot-to-root ratio (Best et al., 1980; Ringwall et al., 2000). The growth of leafy spurge's lateral root buds were suppressed completely at low N levels (Messersmith et al., 1985), while an increase in the N level activated the root buds to resume production (Best et al., 1980), and at high N levels, lateral root production was greatly promoted. In a controlled outdoor experiment, high levels of soil N reduced the biomass of leafy spurge and led to greater root abundance near the soil surface (Ringwall et al., 2000). This shift to producing more roots near the surface could affect leafy spurge in multiple ways: it could increase competition for soil resources with native plants, drought susceptibility, and herbicide translocation to the root system (Ringwall et al., 2000). Thus, N applications can be used to enhance control of leafy spurge.

Liu et al. (2023) found that graminoid P content increased, albeit only marginally, with leafy spurge abundance. Leafy spurge abundance however was not related to soil P levels, suggesting that leafy spurge may alter how graminoids access soil nutrients (Liu et al., 2023). Others have found that P (Arnold, 1984), K, nitrate (NO_3), magnesium (Mg), and sulfate (SO_4^{2-}) were all higher in leafy spurge infested plot areas compared to non-infested areas (Gibbons et al., 2017). They also found that leafy spurge cover was positively correlated with P, NO_3, SO_4^{2-}, Mg, Ca, and K while being negatively correlated with iron (Fe) and manganese (Mn). These findings indicate that leafy spurge directly impacts soil nutrient concentrations, in addition to responding to nutrient availability, and suggest that manipulating nutrients may be an effective avenue for control.

2.4 Invasive Plant Control

Management of invasive plant species can be classed into four main control techniques including cultural, biological, physical or mechanical, and chemical.

2.4.1 Cultural Control

Cultural control involves education to modify human behavior to prevent and limit the spread of invasive species (National Invasive Species Information Center [NISIC], 2024). Prevention is the most effective and economical way to manage invasive species (NISIC, 2024) and includes practices such as cleaning footwear, vehicles, and equipment prior to entry to reduce transportation of seeds and plant materials (St. John & Tilley, 2014). Using certified weed-free seed, hay, straw, and mulch can help prevent introducing invasive plants into new areas (St. John & Tilley, 2014). Overgrazing reduces the competitiveness of desirable grasses, resulting in an increased presence of invasive plants, thus maintaining a healthy native plant community through proper grazing management can also help prevent invasive plant establishment (DiTomaso et al., 2010). Re-establishment of desirable and competitive plant species through revegetation provides a long-term, sustainable method to suppress invasive plants (DiTomaso et al., 2010). Limiting soil disturbance by reducing the size, area, and duration in addition to considering the seasonal timing of the disturbance can improve native vegetation recovery and limit invasive establishment (NISIC, 2024). And, once an invasive plant is identified, early detection and rapid response management is key to minimizing spread.

2.4.2 Biological Control

Biological control includes using natural enemies to reduce, weaken, or kill the host plant (Clay & Scholes, 1997; Johnson et al., 2022). The use of insects (e.g., fleas, beetles, and moths) and prescribed grazing with domesticated herbivores (e.g., cattle, sheep, and goats) are common biological control approaches (NISIC, 2024). Biological control is often used in ecologically sensitive areas, such as riparian areas or shelterbelts, where the application of herbicides or other control options is not ideal (CABI, 2022; Lym, 1998). Insects that eat foliage, flowers, vascular tissues, or roots to reduce the performance or reproductive capacity of the plant are selected (Clay & Scholes, 1997). Insect introductions must undergo rigorous testing and meet certain criteria, such as being host-specific, before approval for release to safeguard against unintended consequences such as attacks on desirable plant species (Clay & Scholes, 1997).

2.4.3 Physical/Mechanical Control

Physical and mechanical control removes the entire plant or physically damages the plant (DiTomaso et al., 2010). Physical control includes labour-intensive techniques such as hand-pulling and digging, while mechanical techniques include mowing and tilling (NISIC, 2024). Appropriately timed mowing can be used to reduce seed production and provide suppression of biennials and perennials when used repeatedly (DiTomaso et al., 2010). Tillage is generally not advised in pastures and rangelands as it can spread perennial invasive plants, especially vegetative reproductive structures such as rhizomes and clonal roots (DiTomaso et al., 2010).

2.4.4 Chemical Control

Chemical control involves the use of pesticides including herbicides and fungicides (NISIC, 2024). Chemical control options can be very effective in managing invasive plant species; however, they can be hazardous to apply, harmful to desired plant species, and damaging to the ecosystem. Applicators must practice careful and selective use of chemical control options and follow label directions for human safety, animal and livestock safety, and the protection of the environment (Johnson et al., 2022). Herbicide use in pastures and rangelands is commonly completed by ground application using backpack sprayers, all-terrain vehicle (ATV) mounted sprayers, and wicks (DiTomaso et al., 2010). In pastures and rangelands, invasive plants often grow in association with other desirable species, hence selecting the appropriate herbicide is important to reduce harm to the desired plant community (DiTomaso et al., 2010). Additionally, application of herbicides can lead to direct and indirect effects on AMF (Hage-Ahmed et al., 2019). Exposure of AMF hyphae, arbuscules, and vesicles to herbicides can alter root colonization with the host plant, though AMF response depends on the active ingredient and the applied dose (Hage-Ahmed et al., 2019). Indirect effects of herbicides on AMF can occur when the herbicide kills the host plant (Hage-Ahmed et al., 2019).

2.5 Leafy Spurge Control

Leafy spurge is extremely difficult to control. Patience, persistence, ongoing monitoring, and evaluation of management techniques are essential for the long-term management of leafy spurge (Gucker, 2010). Management techniques that maintain the integrity of the native plant community while reducing the invasibility of the ecosystem are likely to be more effective than managing the invasive species exclusively (Hobbs & Humphries, 1995).

2.5.1 Cultural Leafy Spurge Control

Grazing management and maintaining overall grassland health is an important cultural control method in preventing invasive plant species (Beck, 2013). Overgrazing can stress grasses making them less competitive and providing an opportunity for invasive species like leafy spurge to establish (Beck, 2013). Proper timing and selection of revegetation with competitive grass species such as western wheatgrass (*Agropyron smithii*) and slender wheatgrass (*Elymus trachycaulus*) can provide high yield and forage nutrition for grazing along with competing with leafy spurge (CABI, 2022; Lym, 1998). Minimizing soil disturbance, ensuring vehicles and equipment are clean prior to entry, and using certified weed-free seed mixes for revegetation can help prevent leafy spurge establishment (U.S. Department of Agriculture, 2001).

2.5.2 Biological Leafy Spurge Control

Several insect species have been approved and released in North America for control of leafy spurge. Many biocontrol agents take time to establish and increase to sufficient numbers to make an impact, meaning biocontrol agents have not kept pace with the spread of leafy spurge (CABI, 2022). The flea beetles (*Aphthona* spp.) have achieved the most success in reducing leafy spurge by attacking the plant in two ways (CABI, 2022; Clay & Scholes, 1997). The larvae feed on the roots of the plant, which reduces water and nutrient uptake and reduces carbohydrate reserves (Clay & Scholes, 1997), while the adults feed on the foliage (CABI, 2022; Lym, 1998). However, the release of the flea beetles in areas where stem density is very high or where the soil is very sandy has seen weak establishment of the beetle (Lym, 1998). Thus, flea beetles cannot solely be relied upon as a successful control agent (Lym, 1998). Releasing beetles between mid-June to mid-July with a 1000 beetles per drop along the edge of the dense leafy spurge patch, as opposed to the center of the dense patch, can help with establishment (Gucker, 2010).

The leafy spurge gall midge (*Spurgia esulae*) has shown success near wooded areas (Lym, 1998) as it requires shelter from the wind (Clay & Scholes, 1997). The gall midge reduces leafy spurge seed production by forming a gall on the terminal ends of shoots (Clay & Scholes, 1997). The larvae of the stem-boring beetle (*Oberea erythrocephala*) mine the roots and stem tissues of leafy spurge (Clay & Scholes, 1997). Released in the early 1980s for leafy spurge control, the population growth of this species is slow and can take several years before it reaches a damaging level (Clay & Scholes, 1997). The spurge hawkmoth (*Hyles euphorbiae*) defoliates leafy spurge, however, this species can be difficult to establish and is not considered prolific enough to provide value (Clay & Scholes, 1997).

Grazing with domestic sheep (*Ovis* spp.) and goats (*Capra* spp.) can be an effective control method in large infestations (Lym, 1998). Grazing should begin early in the spring when leafy spurge first emerges (Lym, 1998). Goats may be preferred to sheep due to dietary overlap concerns with cattle (Lym, 1998). Goats have a 5-20% overlap, while sheep have a 20-35% overlap (Lym, 1998). Additionally, goats accept leafy spurge more readily (Clay & Scholes, 1997) and will graze regardless of the density, while sheep consumption declines as plant density decreases (Lym, 1998). Once seeds are formed, animals must be quarantined for seven days to allow seed passage before moving to a new area (Beck, 2013). Unfortunately, once the animals are removed, leafy spurge regrows to original densities. Even after eight years of intensive grazing with sheep, regrowth from the roots resulted in quick re-establishment one year later (Lym, 1998). In Saskatchewan, a four-year continuous grazing study by domestic sheep demonstrated reduced leafy spurge density on grazed versus ungrazed plots, however, within two years following the removal of the treatment the leafy spurge density was recovered (Bowes & Thomas, 1978).

2.5.3 Physical/Mechanical Leafy Spurge Control

Physical removal of leafy spurge using a shovel was completed in a riparian area where the use of chemicals was not desirable. This method was effective in reducing the length of the main root when digging was completed four times during the growing season (Montemayor et al., 2020). Digging out the main root resulted in fewer stems and also produced stems that were shorter and younger, which resulted in reduced flower and seed production (Montemayor et al., 2020). However, the removal of the leafy spurge plants allowed for another invasive weed, Canada thistle (*Cirsium arvense*), to colonize (Montemayor et al., 2020).

Mowing leafy spurge can be used to remove top growth to prevent seed production and lower root reserves (Johnson et al., 2022), however, mowing must be done at appropriately timed intervals. A study where mowing was completed at two and four week intervals reduced leafy spurge biomass by more than 95% after the first treatment, but once the mowing was stopped, leafy spurge recovered (Clay & Scholes, 1997). Tilling leafy spurge stimulates the development of its root buds and results in an increase in leafy spurge stem density (Messersmith et al., 1985). Thus, tilling as a control method is not recommended (Messersmith et al., 1985), especially in native pastures.

2.5.4 Chemical Leafy Spurge Control

Chemical control using herbicides is often very effective and generates rapid results, making it the most widely used control method for leafy spurge (Clay & Scholes, 1997). Several herbicides are approved for use in native grasslands including 2,4-D, dicamba, and picloram (CABI, 2022), though proper timing of herbicide application is essential for leafy spurge to be susceptible (Lym, 1998). The plant should be in the true flower stage in spring or when the stems have new fall regrowth in early September (Lym, 1998). According to Lym (1998), the distinction between bract appearance and the true flowering parts on leafy spurge is important for appropriately timed herbicide application. Leafy spurge growth in June is characterized by rich green leaves along the entire stem, swelling of the seed capsules, and vigorous plant growth (Lym & Messersmith, 1983). Fall regrowth of leafy spurge is stimulated in late August or early September by cooler weather and rainfall and is characterized by the leafless main stem developing two or more branches below the original flowering branches (Lym & Messersmith, 1983). Although leafy spurge plants may appear to be in poor health in fall, carbohydrates are being transported to the roots for storage in preparation for winter and herbicide treatments have proven to be effective at this time (Lym & Messersmith, 1983). However, to prevent viable seed production, leafy spurge control must be completed prior to the seeds turning brown (Lym, 1998).

Herbicide performance is best when conditions are favourable for plant growth (Johnson et al., 2022) as control declines with low soil moisture and unseasonably warm or cool temperatures (Lym & Messersmith, 1983). In general, herbicides are poorly absorbed by leafy spurge (Lym, 1998) due to the waxy coating on its leaves, thus the addition of a surfactant is often recommended to act as a penetrant (St. John & Tilley, 2014). Leafy spurge also has the ability to purge herbicides from its roots, with more than 60% of the herbicide found in the rhizosphere of the leafy spurge roots (Hickman et al., 1990), further reducing the effectiveness of the herbicide (CABI, 2022). These challenges with herbicide use for control of leafy spurge result in the need for continuous treatments. Careful selection of an effective herbicide along with following the label directions is important for human and animal safety, in addition to environmental considerations (Johnson et al., 2022). However, herbicide use may not always be acceptable due to detrimental effects on associated vegetation, high treatment costs, the potential for groundwater contamination, and other environmental concerns (Gucker, 2010; Lym, 1998).

This research project will focus on chemical control of leafy spurge, specifically investigating the results with four herbicides 2,4-D, Overdrive®, Navius® FLEX, and Tordon 22K used alone and in combination with various fertilizer treatments.

2.6 Herbicides

2.6.1 2,4-D Ester 700

Introduced in the late 1940’s, 2,4-D is one of the oldest and most widely used herbicides worldwide to control broadleaf weeds in grass crops (Joseph et al., 2018; MacDonald et al., 2013; Peterson et al., 2016). 2,4-D is a synthetic growth regulating herbicide that belongs to the chemical group phenoxycarboxylic acids which controls susceptible broadleaf plants by mimicking the plants naturally occurring auxins (Joseph et al., 2018; MacDonald et al., 2013). 2,4-D ester (2,4-D) is a liquid Group 4 selective herbicide with a short half-life of approximately 6 days designed to injure broadleaved plants without causing harm to graminoid species (Petersen et al., 2013). 2,4-D provides suppression and top growth control of many perennial broadleaf weeds (Nufarm Canada, 2019). 2,4-D ester formulations are generally more effective than 2,4-D amine formulations for controlling pasture weeds (Johnson et al., 2022), as esters are better able to penetrate the leaf cuticle resulting in more rapid uptake (Peterson et al., 2016). Thus, ester formulations of 2,4-D produce a more rapid plant response. Additionally, unlike amine formulations, ester formulations do not dissociate or ionize when added to water and therefore do not react with hard water cations to form insoluble precipitates (Peterson et al., 2016). Ester formulations are also considered more rainfast (Peterson et al., 2016). Visual symptoms of impacted plants includes twisting of petioles and leaves, leaf chlorosis, stem tissue proliferation, and abnormal apical growth (Joseph et al., 2018). 2,4-D uptake and translocation is greatest under warm and humid conditions (Petersen et al., 2013). Uptake is also improved with adjuvants and lower pH (Petersen et al., 2013). Conversely, water stress does not affect uptake but does reduce translocation (Petersen et al., 2013).

2,4-D offers limited control of creeping perennials (Deneke et al., 2010; Johnson et al., 2022), as such, only top growth control of leafy spurge can be expected. Lym and Messersmith (1983) found spring applied 2,4-D controlled top growth of leafy spurge but control declined to less than 50 percent by fall and less than 20 percent the following spring.

In grasslands, 2,4-D is often tank mixed with other growth regulator herbicides such as aminopyralid, dicamba, or picloram to improve efficacy on perennials and woody species (Petersen et al., 2013). 2,4-D tank mixed with picloram at 1.1 kg per hectare (kg/ha) and 0.28 to 0.56 kg per hectare (1.0 pound per acre (lb/ac) and 0.25 to 0.5 pound per acre) respectively and applied annually, controlled leafy spurge at 50 to 60 percent after one year and resulted in nearly three times as much forage production following the first application (Lym & Messersmith, 1983).

Re-entry restrictions with 2,4-D include not permitting dairy animals to graze treated areas within 7 days of application with meat animals withdrawn at least 3 days before slaughter (Nufarm Canada, 2019). Grazing or cutting of treated forage should not occur until 30 days after application (Nufarm Canada, 2019).

2.6.2 OVERDRIVE®

OVERDRIVE® (Overdrive) is a Group 4 and 19 wettable granule herbicide manufactured by BASF Corporation and registered for use in pastures and rangelands (BASF Canada Inc., n.d.-b). Overdrive contains two active ingredients Diflufenzopyr (present as sodium salt) - 20% a.e. and Dicamba (present as sodium salt) - 50% a.e. and is a selective post emergent herbicide used to control annual and biennial broadleaf weeds and control or suppress perennial broadleaf weeds in pastures and rangelands (BASF Canada Inc., n.d.-b; Deneke et al., 2010).

Diflufenzopyr is an auxin-transport inhibitor that traps the transport of auxins, the plants natural growth hormones, in the meristems, while dicamba is a synthetic auxin that mimics the natural plant hormones and causes rapid uncontrolled cell division and growth (BASF Canada Inc., n.d.-b; Lym & Deibert, 2005). Thus, when diflufenzopyr is combined with an auxin mimic such as dicamba, the plants hormones become concentrated and activity is increased in the plants meristematic tissues causing the plant to die (BASF Canada Inc., n.d.-b; Lym & Deibert, 2005). Lym and Deibert (2005) found that ^{14}C-dicamba absorption decreased from 60% when applied alone down to 14% when applied with diflufenzopyr, although leafy spurge control was increased when diflufenzopyr was applied with dicamba. The increased control with diflufenzopyr occurs as a result of the diflufenzopyr directing the dicamba to the growing points of the plant (Lym & Deibert, 2005). Consequently, the combination of diflufenzopyr and dicamba achieves faster control at lower rates compared to dicamba alone (BASF Canada Inc., 2018).

Product performance is improved when Overdrive is applied to the active growth stage of broadleaved weeds along with a non-ionic surfactant in combination with a liquid fertilizer (BASF Canada Inc., n.d.-b). MERGE® Adjuvant may be substituted for the non-ionic surfactant and the liquid fertilizer (BASF Canada Inc., n.d.-b). MERGE® adjuvant is a blend of surfactant (50%) with petroleum hydrocarbons (50%) for application with various herbicides, manufactured by BASF Corporation (BASF Canada Inc., n.d.-a). Overdrive provides top growth suppression for leafy spurge applied at a rate of 285 g/ha using sufficient water to cover the targeted weed (BASF Canada Inc., n.d.-b). Symptoms, including twisting and crinkling, are usually visible within several hours with complete control achieved within 3-7 days (BASF Canada Inc., n.d.-b). Diflufenzopyr may injure annual grasses and can reduce regrowth of warm-season grasses resulting in decreased productivity of desirable forage grasses in rangelands (Lym & Deibert, 2005). Grasses may display twisting and be somewhat stunted but will remain green (BASF Canada Inc., n.d.-b).

Re-entry restrictions for Overdrive include not allowing worker entry into the treated area for 12 hours following application (BASF Canada Inc., n.d.-b). Grazing of dairy animals is not recommended until 7 days after application with meat animals withdrawn at least 3 days before slaughter (BASF Canada Inc., n.d.-b). Harvesting of forage for hay should not occur until 30 days after application (BASF Canada Inc., n.d.-b).

2.6.3 Navius® FLEX

Navius® FLEX (Navius) is a Group 2 and 4 wettable granular product manufactured by Envu Canada, formerly Bayer CropScience Inc., and labeled for use in pastures and rangelands. Navius is a selective herbicide designed for extended broad-spectrum brush and broadleaf weed control. Containing two active ingredients, Metsulfuron-methyl (MSM) 12.6% and Aminocyclopyrachlor (AMCP) 39.5%, Navius provides two modes of action, auxinic and acetolactate synthase enzyme (ALS) inhibition, for effective broadleaf weed control and resistance management (Envu Canada, 2023).

MSM is a sulfonyl-urea herbicide used to control or supress several annual, biennial, and perennial broadleaf weeds in pasture and rangelands (Deneke et al., 2010). Sulfonyl-urea herbicides are weak acid compounds that are highly water soluble and are readily absorbed by roots from soil applications and transported via the xylem to the shoots and leaves of the plant (MacDonald et al., 2013). MSM is also absorbed through plant foliage applications where it enters the leaves and stems and is translocated to meristematic tissues (MacDonald et al., 2013). The activity of sulfonyl-urea herbicides are remarkably specific with some species controlled while other are not, even within the same genus (MacDonald et al., 2013). These herbicides also have extended soil activity which contributes to their long-lasting control in grassland systems (MacDonald et al., 2013).

AMCP is a new auxin mimic herbicide in a class of herbicides known as pyrimidine carboxylic acids which are designed to control invasive and noxious weeds in pasture and rangeland areas (Oliveira et al., 2011; Thilmony & Lym, 2017). Several dicot families show sensitivity to AMCP including Asteraceae, Chenopodiaceae, Convolvulaceae, Euphorbiaceae, and Fabaceae (Lindenmayer et al., 2013). AMCP provides both foliar and soil activity with excellent control of many perennial weed species, including leafy spurge (Lym, 2014). ^{14}C-aminocyclopyrachlor absorption is rapid in leafy spurge at 72% 48 hours after treatment, with over 12% of the applied ^{14}C-aminocyclopyrachlor translocated to the roots within 24 hours after treatment (Lym, 2014).

AMCP is structurally similar to picloram, however, differences in their physical and chemical properties exist (Adams & Lym, 2015; Conklin & Lym, 2013). AMCP is ten times more soluble in water than picloram and maintains a stronger soil-binding potential with a sorption coefficient of 28 compared to picloram’s 16 (Conklin & Lym, 2013). AMCP can remain active in the soil for up to two years providing extended perennial weed control. Degraded in the soil by microbes, AMCP’s half life is dependent upon environmental conditions such as soil type, soil moisture, and temperature (Conklin & Lym, 2013). Soils with higher clay content and higher organic matter influence the most rapid AMCP dissipation. Soil temperature and moisture increases also results in increased AMCP dissipation, although soil moisture has less of an impact in sandy soils.

For season-long control of leafy spurge, an application rate of 167 g/ha of Navius is recommended and should be applied with a non-ionic surfactant at 0.25% v/v using a minimum spray volume of 200 L/ha when plants are actively growing, typically mid-May through mid-September (Bayer CropScience Inc., 2020). Navius is absorbed quickly by the leaves, stems, and roots of treated plants, with length of control dependent upon the application rate, the condition and growth stage of the targeted weeds, the environmental conditions, and the density and vigor of competing vegetation (Bayer CropScience Inc., 2020). Temporary stunted height or growth suppression of grasses may also occur.

Re-entry restrictions using Navius include not permitting worker entry into treated areas for a minimum of 12 hours following application (Bayer CropScience Inc., 2020). There are no grazing or haying restrictions for lactating or non-lactating animals (including cattle, horses, sheep, and goats) and as such grazing livestock does not have to be moved before, during, or after application of NaviusFlex when used as directed (Bayer CropScience Inc., 2020).

2.6.4 Tordon™22K

Tordon™ 22K (Tordon) is a liquid Group 4 herbicide manufactured by Corteva Agriscience and provides long-lasting control of perennial and biennial weeds in pastures and rangelands. Tordon is a selective herbicide with picloram as the active ingredient, formulated as potassium salt (240 g/L), which provides soil activity and translocation through root systems providing prolonged periods between treatments (Corteva Agriscience Canada Company, 2022). An application rate of 90 ml per 100 m^2 is recommended for leafy spurge spot treatments provided not more than 50% of a hectare is treated.

Although it is one of the most expensive treatment options (Clay & Scholes, 1997), picloram is often considered the most effective herbicide for control of leafy spurge (Lym & Messersmith, 1991). It is most effective when applied at true-flower stage, typically mid-June, or during re-growth in fall from late August to the first hard frost, often in October (Lym & Messersmith, 1991).

Picloram absorption varies among plant species and is dependent upon air temperature, relative humidity, spray additives, and solution pH (Thompson et al., 1996). Picloram absorption in leafy spurge was greatest when applied during the vegetative growth stage and when relative humidity was high (90 to 95%), increasing absorption from 11 to 34% and shoot and root translocation from 5 to 21% (Lym & Messersmith, 1991). Picloram absorption and translocation were also increased in leafy spurge when the temperature increased 6 or 12º C 24 hours before treatment (Lym & Messersmith, 1991). The pH of the spray solution can also influence herbicide uptake and translocation (Petersen et al., 2013). Thompson et al. (1996) found that buffering the solution to a pH of 5 or 7 and including ammonium sulfate increased picloram absorption greater than either treatment alone. The lower pH improved diffusion of the picloram herbicide through the leaf cuticle by increasing the ratio of undissociated molecules, while the ammonium sulfate facilitated the movement of the picloram into the cytoplasm (Thompson et al., 1996).

In North Dakota, picloram provided 70 to 90 percent control of leafy spurge for 18 to 24 months when applied at 1.1 to 2.2 kg ae/ha (Lym & Messersmith, 1991). In an older study, picloram applied at 2.2 kg/ha provided 90 percent control of leafy spurge one year after treatment, however control decreased progressively over the next two years (Lym & Messersmith, 1983). In the year following treatment, forage yields were nearly doubled in heavily infested pastures. Ranchers and land managers have often favoured picloram due to its long-lasting soil residual of up to three years (Lekberg et al., 2017), however, Clay and Scholes (1997) argue the cost of picloram is not justified even when the resulting increase in forage is considered. Additionally, the use of picloram is limited due to potential for groundwater contamination concerns because it is highly soluble in water and has a relatively long soil half-life (MacDonald et al., 2013).

Re-entry restrictions with Tordon include not allowing worker entry into treated areas for a minimum of 12 hours following application (Corteva Agriscience Canada Company, 2022). Grazing and haying restrictions include not permitting lactating dairy animals to graze treated areas within 7 days after application and not harvesting forage within 30 days after application (Corteva Agriscience Canada Company, 2022). Meat animals should be withdrawn from treated areas at least 3 days prior to slaughter (Corteva Agriscience Canada Company, 2022).

2.7 Herbicide Resistance

The wide and successful use of herbicides over the past 70 years has resulted in the evolution of herbicide resistance in hundreds of invasive plant species with most herbicide groups impacted (Gaines et al., 2020). Invasive plant biology, herbicide application rates, and herbicide mixes all influence the type and rate at which herbicide resistance evolves (Gaines et al., 2020). Herbicide efficacy is dependent upon the quantity of herbicide that enters a plant cell and the length of time its active form remains available to interact with the target site (Gaines et al., 2020). Foliar applied herbicides rely on translocation through the phloem for optimal action (Gaines et al., 2020). Herbicide resistance occurs when translocation from the leaves to the growing points is reduced (Gaines et al., 2020). For foliar-applied herbicides, the chemical must be absorbed through the leaves. For soil-activated herbicides, however, there are no reported instances of herbicide resistance for soil-applied herbicides (Gaines et al., 2020). Both 2,4-D and dicamba are known to have reduced translocation in some resistant species (Gaines et al., 2020).

Research has shown that tank mixing two different herbicide modes of action at the time of application is more effective at reducing resistance (Evans et al., 2016). Using multiple herbicides can also result in synergism. When applied together, each herbicide improves the performance of the other thereby increasing the overall performance versus applying the herbicide alone and reducing resistance development (Joseph et al., 2018). Two herbicides used in this study each contain two modes of action, Navius and Overdrive and, like most rangeland herbicides, both have Group 4 activity (Evans et al., 2016). Herbicides labelled for rangeland and pasture use are limited, thus it is important to preserve the effectiveness of these vegetation management tools. Many new herbicides were developed in the 1960s and 70s, however, in recent years there has been a dramatic reduction in new herbicide registrations (Holm & Johnson, 2009), further highlighting the importance of herbicide stewardship (Gaines et al., 2020). Thus, herbicide synergism is an important tool for the management and control of weeds while reducing the advancement of herbicide resistance (Joseph et al., 2018).

2.8 Integrated Invasive Plant Management

Integrated invasive plant management incorporates sustainability into managing invasive plants by combining cultural, biological, physical, and chemical techniques. The use of a single control method is often not sustainable long-term, therefore, combining control tools is often both necessary and advantageous for improved management outcomes (DiTomaso et al., 2010). Integrated invasive plant management considers the invasive plant species, the availability of biological control agents or grazing animals, and the effectiveness of the control technique (DiTomaso et al., 2010). Environmental concerns such as proximity to riparian areas, trees, and the water table, along with climatic conditions, topography, chemical use restrictions, and the cost of the control method should also be considered (DiTomaso et al., 2010).

Examples of integrated approaches for invasive plant management include chemical application to provide initial control of the invasive plant followed by the introduction of a biocontrol agent (MacDonald et al., 2013). Herbicide treatments following fire, which can be used to reduce ground litter, promote seed germination and plant regrowth, and provide control of undesirable species, are often more efficacious (MacDonald et al., 2013). MacDonald et al. (2014) found that hand pulling as a follow-up to mowing plus herbicide treatment was an effective integrated weed management practice for control of spotted knapweed (*Centaurea* *stoebe* L.). Mowing can be used to reduce competition and seed dispersal from invasive species in advance of herbicide application with hand pulling used as a follow-up to remove small numbers of plants that survive herbicide applications (MacDonald et al., 2014). Using a combination of fertilizers and herbicides is known to influence weed community structure (Cathcart et al., 2004). Nitrogen, for example, has been used as an adjuvant to enhance herbicide efficacy and can alter herbicide efficacy, depending on the N levels, the herbicide used and the target weed species (Cathcart et al., 2004).

2.8.1 Integrated Leafy Spurge Management

Achieving long-term control of leafy spurge is challenging, however, the use of an integrated management plan can provide a more successful and cost-effective solution over the use of a single method alone (Lym, 1998). For example, Johnson et al. (2022) found that mowing leafy spurge in the spring followed by a fall herbicide application worked well. Combining flea beetles with grazing by domestic sheep or goats can be effective as the grazing reduces leafy spurge density which can aid in the establishment of the flea beetle population (CABI, 2022). Herbicide combined with flea beetles or the leafy spurge gall midge also offers better control than either method used alone (Lym, 1998). A three-year study combining goat grazing with fall-applied picloram + 2,4-D reduced leafy spurge density by 98% and maintained control for two years, longer than either method alone (Lym, 1998). Regardless of the method, early detection and treatment is key (Lym, 1998), followed by a consistent and persistent management program (Beck, 2013). A successful long-term management plan should include a combination of herbicides, insects, grazing, along with seeding competitive desired species (Lym, 1998).

2.9 Forage Quality and Quantity

Native grasslands and pastures are important resources for livestock producers who use these lands to feed their animals (Katoch, 2023c). Forage quality and quantity are influenced by several biophysical factors including soil, vegetation type and stage of growth, climate, and pasture management practices including herbicide or fertilizer application (Faji et al., 2022; Katoch, 2023c). The nutritional quality of the grassland largely depends on the composition of the forage species and can be affected by invasive plants (Katoch, 2023c).

The nutritional value of forage is defined by the amount of nutrition that can be obtained along with the concentration of any toxic compounds that could reduce animal performance or health (Hancock et al., 2014). Factors that influence forage quality include palatability, intake, digestibility, nutrient content, anti-quality factors, and animal performance (Katoch, 2023b). Forage quality is also influenced by forage species and the stage of maturity at harvest (DePeters, 1993). As plants mature, they become more fibrous, and neutral detergent fiber (NDF) concentration increases (Katoch, 2023a). Thus, forage quality declines with maturity and is the most important factor determining forage quality of a particular species (Ayyadurai et al., 2013).

The nutritional value of forage is estimated by measuring the protein content, and level of digestible carbohydrates and proteins, in addition to lignin, cellulose, and crude fiber contents (Katoch, 2023c). Forage quality influences the digestibility and voluntary intake by livestock, however, the presence of anti-quality factors affects the digestibility of the forage (Katoch, 2023c). Anti-quality factors includes compounds such as tannins, nitrates, alkaloids, cyanogenic glycosides, oxalates, estrogens, and mimosine (Ayyadurai et al., 2013; Katoch, 2023c). The presence and toxicity of these compounds depends on the plant species, environmental conditions, animal sensitivity, and amount consumed (Ayyadurai et al., 2013; Katoch, 2023c).

Detergent fiber analysis includes acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Katoch, 2023a). NDF estimates the total cell wall constituents including hemicellulose, while ADF represents cellulose and lignin (Katoch, 2023a; Schroeder et al., 2023). NDF predicts intake potential while ADF calculates digestibility. As the ADF or lignified cellulose content increases the digestibility of the forage decreases (DePeters, 1993; Schroeder et al., 2023). NDF or cell wall content is associated with the dry matter intake of the forage, thus as the NDF content increases, dry matter intake decreases (DePeters, 1993). Protein is a key nutrient commonly measured as crude protein (CP) which is the N content of the forage times 6.25 (Schroeder et al., 2023).

2.9.1 Forage Quality and Quantity and Invasive Plants

Invasive plants compete with forage species for light, water, and soil nutrients resulting in reduced yield, quality, and palatability of forage grasses for livestock to graze (Faji et al., 2022). Invasive plant species can also impact grazing patterns, poison livestock, increase costs to manage and produce livestock, and reduce land values (DiTomaso, 2000). An empirical model prediction from Rinella and Luschei (2007) indicated that leafy spurge reduces carrying capacity for cattle by 50,000 to 217,000 animals a year and reduces land value by $8-$34 million (US) dollars a year for the 17 state area west of Minnesota to Texas. The effects of leafy spurge on forage quality are not well understood, although nutrient concentrations may be increased in the remaining forage (Liu et al. 2023).

2.9.2 Forage Quality and Quantity and Herbicides

Unlike cropland, where all plants, except the crop, are considered weeds, native grasslands commonly only have one or a few invasive plant species that are the target of control measures and these species are often growing in association with desired plant species (DiTomaso et al., 2013). Therefore, selectivity of herbicides is critical in native grasslands as most herbicides are selective only within certain rates, environmental conditions, and methods of application (DiTomaso et al., 2013). Funderburg and Biermacher (2010) found forage quality is generally not affected by herbicide use, while Payne et al. (2010) found crude protein concentration was higher in nontreated control plots compared with the herbicide treated biomass. Conversely, Israel et al. (2016) reported increased forage quality where metsulfuron was applied alone or in combination with aminocyclopyrachlor or aminopyralid. In terms of effects of herbicide on forage yield, grasses generally show high resilience towards broad-leaf targeted herbicide treatments (Petersen et al., 2013). However, Payne et al. (2010) found that biomass yields were greater in nontreated control plots compared with herbicide treated plots, though Payne suggests the reduced forage quantity was a result of the removal of the legumes with the herbicide treatments. Contrary to Payne, others, including Cason and Sleugh (2023) and Sheley et al. (2000), have found increased productivity following herbicide treatments leading to increased carrying capacity. These findings suggest that forage quality and quantity and herbicide use may be dependent on or be influenced by the herbicide used, the application rate, method, or timing, or environmental conditions.

2.9.3 Forage Quality and Quantity and Fertilizers

Fertilization can improve forage quality and quantity in addition to enhancing the competitive ability of the forage species against invasive plant species (Bork et al., 2007). Fertilization of grasses with N often increases yield and increases crude protein levels, however, fertilization generally has little to no effect on digestibility (Ayyadurai et al., 2013; Dindová et al., 2019). Nitrogen fertilization of a pasture in Turkey showed significant increases in yield response and crude protein content while also decreasing NDF and ADF (Balabanli et al., 2010). Springer

(2021) found low rates of N fertilization increased forage production, although an increase in undesired species was also noted. Thus, it is important to investigate whether the rewards of fertilization are worth the economic and environmental costs.

Although N is commonly identified as a key element in forage productivity, the co-limiting effects of N and P are increasingly being recognized (Fay et al., 2015). For instance, the combined application of N and P increased forage yield by an average of 40% over controls, an increase greater than the sum of the individual additions (Fay et al., 2015). Keller et al. (2023) and Funderburg and Biermacher (2010) also found support for both N and P increasing aboveground biomass. Balabanli et al. (2010) found that N+P fertilization reduced NDF and ADF concentrations lower than that of N fertilization alone. Radujković et al. (2021) found two micronutrients, Fe and Zn, were significantly associated with variation in grassland biomass. Fay et al. (2015) findings suggest that K and micronutrients also contribute to grassland productivity, though fertilization with P, K, or other nutrients usually reduces forage quality, especially when plant growth is rapid (Ayyadurai et al., 2013). As with herbicides, the effects of fertilizers on forage quality are thus strongly context dependent and variable among quality indicators.

Chapter 3: Herbicide and Fertilizer Treatment Effects on Leafy Spurge Control and Plant Community Composition and Diversity

3.1 Introduction

Introduced from Eurasia in the early 1800s, leafy spurge [*Euphorbia virgata* Wald & Kit (previously known as *Euphorbia esula* L.) see Flora of North America (Riina et al., 2020)] is an aggressive invasive forb found throughout North America with a strong presence in the Canadian Prairie provinces (Best et al., 1980). Because of its vigorous clonal character and strong competitive abilities, leafy spurge contributes to the degradation of the native ecosystem by reducing indigenous plant richness and diversity and decreasing grassland productivity (Liu et al., 2023; Messersmith et al., 1985). Additionally, leafy spurge can cause changes in the soil as a result of modifications in the biotic and abiotic structures, including shifts in the soil microbial communities and alterations to nutrient availability (Gibbons et al., 2017). As such, leafy spurge is listed as one of the worst noxious weeds in Canada and the United States (Gucker, 2010). It is also extremely challenging to control (Lym, 1998).

Management actions to control invasive plant species, including leafy spurge, often have negative side effects and may be as harmful to the resident plant community (Rinella et al., 2009). Alternatively, these negative effects may be offset by positive effects from the native species as they rebound following management suppression efforts (Rinella et al., 2009). Thus, invasive plant management can have both positive and negative consequences for the native plant community making it challenging for land managers to ascertain whether control efforts are worthwhile (Rinella et al., 2009).

Herbicides used in native grasslands are often selected to target one or more invasive plants while attempting to maintain or improve the native plant community (Crone et al., 2009). However, broadleaf herbicides can negatively impact native forb species and shift the plant community toward grasses (Crone et al., 2009). Repeated use of a single herbicide can select for resistance in the target weed species in addition to causing population shifts and soil nutrient changes that may alter the health of the range ecosystem (DiTomaso, 2000). Undesirable plant species, such as invasive grasses, may replace the invasive forb controlled with the broadleaf selective herbicide (Cole et al., 2007). Herbicides can also impact important plant symbionts like arbuscular mycorrhizal fungi (AMF) by killing its host plant (Lekberg & Gibbons, 2016). Moreover, herbicide use may not always be appropriate due to detrimental effects to native vegetation, water contamination concerns, safety concerns to humans or animals, or high treatment costs (Gucker, 2010; Johnson et al., 2022; Lym, 1998).

Fertilizers are another tool that can be used to increase plant competition and enhance competitive suppression of invaders by native species (Cole et al., 2007). Conversely, fertilizer application may enhance the invasive species growth over the desired native plant growth, especially when the invasive plant species density is high (Cole et al., 2007). Nutrient additions in grasslands can also lead to a loss of plant diversity and shift the composition of the plant community to faster growing plants (Leff et al., 2015). Thus, enhanced soil nutrient availability may enable invasive plants to establish over the native plants (Akin-Fajiye et al., 2021). Furthermore, soil microbial communities including AMF are sensitive to nutrient additions (Leff et al., 2015). A long-term study by Wu et al. (2022) found that P and Zn reduced soil fungal diversity, including AMF. Lekberg et al. (2021) observed that P suppressed AMF but only when P was added together with N. Leafy spurge associates strongly with AMF in a symbiotic relationship, however, in a nutrient-rich environment this relationship can switch and the AMF can become parasitic to the host plant (Lekberg et al., 2021). Thus, the use of fertilizer may be an effective tool in controlling leafy spurge by reversing the AMF association from beneficial to parasitic.

Given that leafy spurge is so challenging to control (Lym, 1998), herbicides are often the management tool of choice because they are effective and deliver rapid results (Clay & Scholes, 1997). However, herbicides alone seldom provide long-term control (DiTomaso, 2000). Combining two or more control techniques can improve sustainability and minimize risk to the environment (National Invasive Species Information Center, 2024), while providing a more successful and cost-effective solution over a single method alone (Lym, 1998). This strategy is known as integrated invasive plant management. Integrating the effects of herbicides with fertilizers is known to influence invasive plant community structure (Cathcart et al., 2004). While the herbicide works to control the invasive plant, the fertilizer aims to increase native plant competition (Cole et al., 2007), in addition to altering the leafy spurge AMF association from beneficial to parasitic (Lekberg et al., 2021). Thus the integration of fertilizers with herbicides compliment each other and can improve long-term control over use of either herbicide or fertilizer alone (Cole et al., 2007). However, the combined effects of herbicides and fertilizers on rangeland soils and plant communities may lead to unintended consequences; as such, investigation is needed.

The goal of this study is to improve herbicide efficacy on leafy spurge in combination with fertilizer and create a more sustainable approach by reducing dependency on herbicides alone. We hypothesize that phosphorus (P), and micronutrient fertilizers, particularly zinc (Zn), will minimize leafy spurge abundance in the nutrient rich environment by reversing the AMF relationship from beneficial to parasitic, especially when combined with herbicide application. The P and Zn fertilizers will reduce AMF colonization with leafy spurge and in doing so will decrease leafy spurge’s ability to acquire soil nutrients, further improving control of leafy spurge (Wu et al., 2022).

3.2 Materials and Methods

3.2.1 Study Sites

We selected four sites for the project: St. Denis, Grenfell, North Battleford, and the Manitou Community Pasture located south of Marsden, SK (**Table 3.1**). These sites are all native prairie grassland, and had sufficient leafy spurge for the research blocks, along with willing participation of the landowner(s).

Table 3.1: Research block locations.

Site	Land Location	Block	Latitude	Longitude
St. Denis	SE-10-038-01 W3M	1	52° 14’53.95” N	106° 3’41.59” W
St. Denis	SE-10-038-01 W3M	2	52° 14’54.81” N	106° 3’37.83” W
St. Denis	SE-10-038-01 W3M	3	52° 15’8.54” N	106° 3’19.81” W
North Battleford	SE-03-043-16 W3M	4	52° 40’7.71” N	108° 14’2.21” W
North Battleford	SE-03-043-16 W3M	5	52° 40’6.43” N	108° 13’57.40” W
North Battleford	SE-03-043-16 W3M	6	52° 40’24.39” N	108° 14’26.06” W
Grenfell	NE-08-016-07 W2M	7	50° 20’4.29” N	102° 55’39.76” W
Grenfell	NE-08-016-07 W2M	8	50° 20’5.64” N	102° 55’35.37” W
Grenfell	NE-08-016-07 W2M	9	50° 20’7.40” N	102° 55’35.59” W
Manitou	SE-04-043-27 W3M	10	52° 40’17.18” N	109° 51’7.60” W
Manitou	SE-04-043-27 W3M	11	52° 40’16.34” N	109° 51’7.28” W
Manitou	SE-04-043-27 W3M	12	52° 40’16.32” N	109° 51’6.31” W

3.2.1.1 St. Denis

The St. Denis site (SE-10-038-01 W3M) is in the RM of Grant # 372, approximately 38 km east and 13 km north from Saskatoon, SK. These Fish and Wildlife Development Fund (FWDF) lands are managed to preserve wildlife habitat. Historically, the site has had limited grazing until 2020 when conservation grazing was introduced (C. Olson, personal communication, June 6, 2022).

Ecologically, the St. Denis site lies within the Minichinas Upland Landscape Area within the Moist Mixed Grassland Ecoregion of the Prairie Ecozone (Saskatchewan Conservation Data Centre, 2014b). The Minichinas Upland is a hilly morainal upland located east of Saskatoon, SK, with elevations between 530 m to 600 m (Acton et al., 1998) characterized by semi-arid moisture conditions and dark brown chernozemic soils with loam surface texture (Saskatchewan Conservation Data Centre, 2014b; SKSIS Working Group, 2018). The landscape of the area is comprised of short, steep slopes with numerous undrained depressions or sloughs (Saskatchewan Conservation Data Centre, 2014b). Native vegetation includes speargrasses (*Hesperostipa* spp.), wheatgrasses (*Elymus* spp. and *Pascopyrum smithii*), and deciduous shrubs including snowberry (*Symphoricarpos* spp.), rose (*Rosa* spp.), chokecherry (*Prunus virginiana*), and wolf willow (*Elaeagnus commutata*), with small aspen (*Populus tremuloides*) groves around sloughs (Saskatchewan Conservation Data Centre, 2014b).

The St. Denis area has a subhumid continental climate with a mean annual daily temperature of 2.4ºC, a mean temperature in July of 18.4ºC, and a mean January temperature of -16.7ºC (Acton et al., 1998). Mean annual precipitation amount is 383 mm, with rainfall from May to September accounting for 240 mm (Acton et al., 1998). Summers are short and warm with a frost-free period of 110 days (Acton et al., 1998). Weather data (Government of Canada, 2023) including mean average monthly temperature, maximum average monthly temperature, minimum average monthly temperature, and total monthly precipitation for the period from May 2022 to July 2023 is included in **Table 3.2**. Total precipitation for the 2022 period of the experiment was 169.2 mm[♦], with 134.4 mm[♦] received for the 2023 period.

[♦] Indicates value is based upon incomplete data.

Table 3.2: Weather data for St. Denis from May 2022 to July 2023.Weather data obtained from the nearest weather station at Saskatoon RCS, SK (Climate ID: 4057165) (Government of Canada, 2023).

Month-Year	Max Monthly Avg. Temp. (ºC)	Min Monthly Avg. Temp. (ºC)	Mean Monthly Avg. Temp. (ºC)	Total Monthly Precipitation (mm)
May-22	18.6	3.5	11.0	25.8*
Jun-22	22.6	8.8	15.7	38.0
Jul-22	26.5	12.0	19.3	46.5
Aug-22	27.9	11.4	19.6	25.6
Sep-22	23.4	6.6	15.0	6.8
Oct-22	14.2	-0.1	7.1	5.1
Nov-22	-4.3	-13.2	-8.8	8.8
Dec-22	-14.2	-23.3	-18.8	12.6
Jan-23	-8.3	-16.8	-12.5	5.0
Feb-23	-8.5*	-19.4*	-14.0*	4.5*
Mar-23	-6.4	-17.7	-12.1	1.1
Apr-23	7.4	-4.5	1.4	8.2
May-23	23.2*	7.8*	15.5*	51.9*
Jun-23	26.6	11.8	19.2	43.8
Jul-23	25.1	10.3	17.7	19.9

** Value based on incomplete data.*

3.2.1.2 North Battleford

The North Battleford site (SE-03-043-16 W3M) is in the RM Battle River #438 approximately 15 km south of North Battleford, SK. The land is Sweetgrass First Nation Reserve Land and occupied by Triple T Farm under the direction of brothers Todd and Trevor Buchko. The land is grazed annually with cattle.

Ecologically, the North Battleford site lies within the Lower Battle River Landscape Area within the Aspen Parkland Ecoregion of the Prairie Ecozone (Saskatchewan Conservation Data Centre, 2014a). The Lower Battle River Plain is a hummocky sand plain between North Battleford, SK and Maidstone, SK, with an elevation of approximately 550 m (Acton et al., 1998). The hummocky landscape has moderate slopes of 5-15%, with regosolic soils formed in wind-worked, sandy fluvial materials in the black soil zone with loamy sand surface texture (SKSIS Working Group, 2018). Because of the sandy soils in the area, there is a high percentage of uncultivated land with most of the area in rangeland or pasture (Acton et al., 1998). The Ecoregion is characterized by a mosaic of trembling aspen (*Populus tremuloides*), found on the moist lower, mid- and north-facing slopes, and fescue grasslands, on the drier upper and south-facing slopes (Saskatchewan Conservation Data Centre, 2014a).

The North Battleford area has a humid continental climate with a mean annual daily temperature of 1.4ºC, a mean temperature in July of 18.0ºC, and a mean January temperature of -18.9ºC (Acton et al., 1998). Mean annual precipitation amount is 420 mm, with rainfall from May to September accounting for 262 mm (Acton et al., 1998). Summers are short and warm with a frost-free period of 106 days (Acton et al., 1998). Weather data (Government of Canada, 2023) including mean average monthly temperature, maximum average monthly temperature, minimum average monthly temperature, and total monthly precipitation for the period from May 2022 to July 2023 is included in **Table 3.3**. Total precipitation for the 2022 period of the experiment was 258.3 mm♦, with 123.0 mm♦ received for the 2023 period.

Table 3.3: Weather data for North Battleford from May 2022 to July 2023.Weather data obtained from the nearest weather station at North Battleford, SK (Climate ID: 4045695) (Government of Canada, 2023).

Month-Year	Max Monthly Avg. Temp. (ºC)	Min Monthly Avg. Temp. (ºC)	Mean Monthly Avg. Temp. (ºC)	Total Monthly Precipitation (mm)
May-22	18.2	2.8	10.5	26.2
Jun-22	21.3*	8.7*	15.0*	133.1*
Jul-22	25.6*	11.7*	18.7	49.1*
Aug-22	27.2	10.9	19.1	30.5
Sep-22	23.1	5.7	14.4	4.9
Oct-22	14.2*	-1.1*	6.5*	1.7*
Nov-22	-5.5	-14.1	-9.8	12.2
Dec-22	-14.8	-23.1	-19.0	0.6*
Jan-23	-8.7	-16.6	-12.7	3.8
Feb-23	-7.7	-17.7	-12.7	0.7
Mar-23	-6.7	-17.2	-12.0	0.4
Apr-23	9.1*	-3.5*	2.8*	7.5*
May-23	23.2	7.5	15.3	32.3
Jun-23	25.6	10.5	18.1	40.2
Jul-23	24.4*	9.9*	17.2*	38.1*

** Value based on incomplete data.*

♦ Indicates value is based upon incomplete data.

3.2.1.3 Grenfell

The Grenfell site (NE-08-016-07 W2M) is in the RM of Elcapo #154 approximately 10 kilometers south of the community of Grenfell, SK. The land is privately owned by Bill and Colleen Kent and has not been grazed for the last few years, nor was it grazed during the 2022 or 2023 season.

Ecologically, the Grenfell site is located within the Kipling Plain Landscape Area within the Aspen Parkland Ecoregion of the Prairie Ecozone (Saskatchewan Conservation Data Centre, 2014a). The Kipling Plain has elevations of 600 m to 700 m with surface drainage to the Qu'Appelle River through several creeks including the Pipestone Creek (Acton et al., 1998) which runs through the southern portion of the Grenfell site. The Pipestone Creek valley provides native vegetation cover with black loam soils on moderately sloping hummocky moraines (Acton et al., 1998).

The Grenfell area has a humid continental climate with a mean annual daily temperature of 1.4ºC, a mean temperature in July of 18.0ºC, and a mean January temperature of -18.9ºC (Acton et al., 1998). Mean annual precipitation amount is 420 mm, with rainfall from May to September accounting for 262 mm (Acton et al., 1998). Summers are short and warm with a frost-free period of 106 days (Acton et al., 1998). Weather data (Government of Canada, 2023) including mean average monthly temperature, maximum average monthly temperature, minimum average monthly temperature, and total monthly precipitation for the period from May 2022 to July 2023 is included in **Table 3.4**. Total precipitation for the 2022 period of the experiment was 347.1 mm[♦], with 130.0 mm[♦] received for the 2023 period.

[♦] Indicates value is based upon incomplete data.

Table 3.4: Weather data for Grenfell from May 2022 to July 2023.Weather data obtained from the nearest weather station at Indian Head CDA, SK (Climate ID: 4013480) (Government of Canada, 2023).

Month-Year	Max Monthly Avg. Temp. (⁰C)	Min Monthly Avg. Temp. (⁰C)	Mean Monthly Avg. Temp. (⁰C)	Total Monthly Precipitation (mm)
May-22	17.5*	4.0*	10.7*	97.7*
Jun-22	23.5	8.6	16.1	27.5
Jul-22	24.4	11.8	18.1	114.5
Aug-22	25.3	11.3	18.3	45.9
Sep-22	22.1	5.4	13.7	14.5
Oct-22	12.4	-1.2	5.6	18.8
Nov-22	-3.3*	-12.6*	-8.0*	8.5*
Dec-22	-13.0	-21.7	-17.3	19.7
Jan-23	-7.8	-15.7	-11.8	1.3
Feb-23	-7.4	-18.4	-12.9	7.0
Mar-23	-6.5	-19.0	-12.7	6.7
Apr-23	4.6	-5.6	-0.5	36.6
May-23	22.4	5.6	14.0	12.9
Jun-23	26.7	12.2	19.4	49.6
Jul-23	24.9	8.5	16.7	15.9

** Value based on incomplete data.*

3.2.1.4 Manitou

The Manitou site (SE-04-043-27 W3M) is in the RM of Manitou Lake #442 located 5 km west and 20 km south from the village of Marsden, SK. The land is Saskatchewan Agricultural Crown owned and operated by the Manitou Cattle Breeders Co-op and grazed annually with cattle.

Ecologically, the Manitou site is located within the Ribstone Plain Landscape Area within the Aspen Parkland Ecoregion of the Prairie Ecozone (Saskatchewan Conservation Data Centre, 2014a). The Ribstone Plain is a small area that include the Manitou sand hills with elevations between 600 m and 700 m (Acton et al., 1998). This sandy area has a high proportion of uncultivated land with most of the area being rangeland and pasture occupied by several large grazing cooperatives (Acton et al., 1998). There is extensive cover of native vegetation including trembling aspen (*Populus tremuloides*) woodlands, although the rougher dunes consist of more open grassland (Acton et al., 1998). The weakly developed dark brown regosolic soils with fine sand surface texture have been altered by wind erosion to form the hummocky ridged relief in the area (Acton et al., 1998; SKSIS Working Group, 2018). The Manitou sand hills, with its unique surficial geology, has a rare mix of plant communities and as such has been designated an Important Plant Area which supports in excess of 370 vascular plant species (Godwin, 2019).

The Manitou area has a humid continental climate with a mean annual daily temperature of 1.4°C, a mean temperature in July of 18.0°C, and a mean January temperature of -18.9°C (Acton et al., 1998). Mean annual precipitation amount is 420 mm, with rainfall from May to September accounting for 262 mm (Acton et al., 1998). Summers are short and warm with a frost-free period of 106 days (Acton et al., 1998). Weather data (Government of Canada, 2023) including mean average monthly temperature, maximum average monthly temperature, minimum average monthly temperature, and total monthly precipitation for the period from May 2022 to July 2023 is included in **Table 3.5**. Total precipitation for the 2022 period of the experiment was 213.1 mm[♦], with 177.2 mm[♦] received for the 2023 period.

Table 3.5: Weather data for Manitou from May 2022 to July 2023.Weather data obtained from the nearest weather station at Lloydminster, AB (Climate ID: 3013959) (Government of Canada, 2023).

Month-Year	Max Monthly Avg. Temp. (°C)	Min Monthly Avg. Temp. (°C)	Mean Monthly Avg. Temp. (°C)	Total Monthly Precipitation (mm)
May-22	16.9*	2.8*	9.8*	44.3*
Jun-22	20.8	8.7	14.8	93.1
Jul-22	23.7*	11.5*	17.6*	40.1*
Aug-22	25.3*	11.5*	18.4*	23.2
Sep-22	21.5	5.4	13.5	3.2
Oct-22	14.2*	0.3*	7.3*	0.4*
Nov-22	-5.4	-12.3	-8.8	6.7
Dec-22	-14.7	-22.7	-18.7	2.1
Jan-23	-7.1*	-14.1*	-10.6*	3.2*
Feb-23	-7.3	-16.8	-12.1	0.2
Mar-23	-5.7	-15.5	-10.6	0.0
Apr-23	7.9*	-3.1*	2.4*	11.6*
May-23	22.8	7.7	15.3	7.3
Jun-23	23.5	11.5	17.5	116.7
Jul-23	22.8	10.9	16.9	38.2

** Value based on incomplete data.*

[♦] Indicates value is based upon incomplete data.

3.2.2 Experimental Design

During the 2022 growing season we established a randomized complete block design experiment with all treatments applied by individual plot. Four different herbicides [5 treatments = Control + 4 herbicides (2-4D Ester 700 (2,4-D), Navius® FLEX (Navius), OVERDRIVE® (Overdrive), and Tordon^{TM} 22K (Tordon))] along with various fertilizers [6 treatments = Control + 5 fertilizers (nitrogen (N), phosphorus (P), micronutrient mix (micro) (Micromax® contained the following micronutrients: Calcium (Ca), Magnesium (Mg), Sulfur (S), Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), and Zinc (Zn) (**Table 3.6**)) were applied alone and in combination (Control, N, P, N+P, micro, N+P+Micro))] across four sites in Saskatchewan, in two different soil zones (Black and Dark brown soil zones) and two different soil textures (loamy and sandy). Three replicate blocks (270 m^2 each) per site were established for the project with treatment plot sizes of 3 m x 3 m, for a total of 360 plots (4 sites x 3 replicates x 30 treatment plots) (**Table 3.7**). A 1 m x 1m buffer was placed around each plot to account for treatment spillover and edge effects. All plots were staked with 0.9 m (36 inch) pigtail markers located in each corner, a plot tag and herbicide treatment flags were attached to the southeast corner marker, with fertilizer treatment flags placed on the northeast corner. A Trimble GPS point was taken at each plot tag location. Electric fence was set up around each block, except at the Grenfell site where the landowner was not grazing the pasture. Plot setup was completed between June 7-17, 2022.

Herbicide treatment was intended to be applied once in year one as we wished to explore the residual effects of some of the herbicides; however, an application error resulted in a couple herbicide applications being repeated (details discussed in following paragraph). Herbicide application was applied during the vegetative to flowering stage of growth of the leafy spurge and was completed using backpack sprayers with each herbicide allocated to its own dedicated sprayer and applied at manufacturer recommended rates. 2,4-D was applied at an application rate of 3.4 L/ha (660 g a.e./L) , Navius (Aminocyclopyrachlor (AMCP) + Metsulfuron-methyl (MSM)) application rate was 167 g/ha (AMCP @ 66 g a.e./ha and MSM @ 21 g a.e./ha) with Hasten NT nonionic surfactant at a rate of 0.25% (v/v), Overdrive (Diflufenzopyr (Df) + Dicamba (Di)) application rate was 285 g/ha (Di @ 142.5 g a.e./ha and Df @ 57 g a.e./ha) with MERGE® nonionic surfactant at a rate of 0.25% (v/v), and Tordon (picloram) was applied at an application rate of 9 L/ha (240 g a.e./L) (**Table 3.8**).

Table 3.6: Micromax® micronutrients and guaranteed analysis.

Micronutrient	Guaranteed Analysis (%)
Calcium (Ca)	6.00
Magnesium (Mg)	3.00
Sulfur (S)	12.00
Boron (B)	0.10
Copper (Cu)	1.00
Iron (Fe)	17.00
Manganese (Mn)	2.50
Molybdenum (Mo)	0.05
Zinc (Zn)	1.00

Table 3.7: Herbicide and fertilizer treatment matrix.

	Herbicides				
Fertilizers	**Control**	**2,4-D**	**NaviusFlex**	**Overdrive**	**Tordon 22K**
	N	N + 2,4-D	N +Navius	N + Overdrive	N + Tordon
	P	P + 2,4-D	P + Navius	P + Overdrive	P + Tordon
	micro	micro + 2,4-D	micro + Navius	micro + Overdrive	micro + Tordon
	N+P	N+P + 2,4-D	N+P + Navius	N+P + Overdrive	N+P + Tordon
	N+P+Micro	N+P+Micro + 2,4-D	N+P+Micro + Navius	N+P+Micro + Overdrive	N+P+Micro + Tordon

Table 3.8: Herbicide application rate.

Herbicide	Acid Equivalent (a.e.)	Application Rate	Surfactant
2,4-D	660 g/L	3.4 L/ha	N/A
Navius		167 g/ha	Hasten NT 0.25% (v/v)
AMCP	66 g/ha		
MSM	21 g/ha		
Overdrive		285 g/ha	Merge 0.25% (v/v)
Di	142.5 g./ha		
Df	57 g/ha		
Tordon (picloram)	240 g/L	9 L/ha	N/A

Herbicide application was completed over each plot with the first pass made in one direction and a second pass made in an opposing direction to ensure complete herbicide coverage. All six (6) plots were sprayed with any remaining herbicide solution such that all herbicide was sprayed out within that block. 2,4-D was applied at a double rate in Block 1 at St. Denis, where it was established that the amount of calculated water was not sufficient to provide adequate coverage across the six (6) treatment plots within the block. The water amount was increased for all subsequent herbicide applications at all remaining blocks, however, the surfactant amount (a volume-to-volume ratio) for Navius and Overdrive was inadvertently not increased, and as such, an insufficient amount of surfactant was applied. This oversight was not recognized until after completion of spraying at all blocks. Poor efficacy was subsequently observed with Navius and Overdrive, so we re-applied both herbicides using the correct surfactant amount for the volume of water following the initial soil and biomass collection in late July and early August. The leafy spurge was at an advanced stage of maturity for this application.

Fertilizer treatments were applied by hand (**Table 3.9**). Due to a calculation error, N and P treatments were not applied at the desired amount in 2022. This error was corrected in 2023, with the desired amount of fertilizer was applied. Additionally, a few fertilizer misapplications occurred in 2022, and one application error in 2023, resulting in the wrong fertilizer being applied to a plot, primarily with micronutrient fertilizer [n=22 (Control = 1, micro = 10, N+P+Micro = 11)]. Fertilizer application errors were excluded from all statistical analyses.

Table 3.9: Fertilizer application rates for 2022 and 2023.

Fertilizer	**2022**	**2023**
N	52 kg/ha (46 lbs/ac)	112 kg/ha (100 lbs/ac)
P	13 kg/ha (11.6 lbs/ac)	67 kg/ha (60 lbs/ac)
micro	97.5 kg/ha (87 lbs/ac)	97.5 kg/ha (87 lbs/ac)

We measured annual changes in leafy spurge abundance and plant composition in both 2022 and 2023. Leafy spurge was measured as percent cover in the 0.25 m^2 quadrat in both years and in the 3 m x 3 m treatment plot in 2023. At the same time, plant composition was measured as percent cover of all vascular plants in the 0.25 m^2 quadrat in both years. In 2022, data were collected from July 19 to August 10, and, in 2023, data were collected from July 6 to July 19.

For 2022 leafy spurge densities were too low in herbicide plots to estimate colonization, so we collected soil samples from each plot to determine the effects of the treatments on mycorrhizal colonization of trap plants. Two (2) soil cores (5 cm diameter x 15 cm depth) were collected from the biomass subplot once the vegetation was collected and placed in a separate labelled sealable plastic storage bag. For 2023, once leafy spurge began to recover in some of the herbicide plots, we collected leafy spurge roots from each treatment plot and assessed these for colonization.

In 2022, plant-root simulator probes (PRS® probes from Western Ag) were used to measure changes in the soil nutrient (NO_3, NH_4, P, K, S, Ca, Mg, Al, Fe, Mn, Cu, Zn, B, Pb, and Cd) availability to verify treatment effects on soil nutrients. These probes were deployed for 42-61 days, then collected for analysis. Probes were cleaned with distilled water using a small toothbrush to remove soil particles, then returned to the Western Ag lab for analysis.

3.2.3 AMF Colonization Estimation

To determine the effects of the herbicide and fertilizer treatments on mycorrhizal colonization, we assessed 2022 AMF colonization via a phytometer trial using field collected soils. Soil from each plot was placed individually in a 50 ml Falcon® tube along with five (5) seeds of sorghum-sudangrass (Sorghum × drummondii (Nees ex. Steud.) Millsp. & Chase) which were planted at a depth of 2.5 cm and grown in the University of Saskatchewan's phytotron for two (2) weeks. Sorghum is an important cereal crop that is highly colonized by AMF (Watts-Williams et al., 2022), making it a good choice for a colonization assay. The phytotron chamber temperature was 22 ^{o}C with 16 hours of light and 18 ^{o}C with 8 hours of darkness per day. Plants were watered three times a week with approximately 10 ml of water at each watering. Following the two-week growing period, the plants were harvested, and their roots were washed and stored in a 70% ethanol solution. Five roots from each sample were selected and subjected to a staining process where they were placed in a 95°C bath, firstly submersed in a 10% potassium hydroxide (KOH) solution for two (2) hours; followed by a 2% hydrochloric acid (HCl) solution for 30 minutes; and finally, a 0.1 g/L trypan blue solution for 30 minutes. After this, they were placed in glass jars filled with distilled water and 20 drops of acetic acid to destain for at least 24 hours and kept cool in a fridge. After destaining, the roots were placed in glass slides and covered with a toluene-based mounting medium. The slides were then examined under a microscope for assessment of percentage colonization using the line intersect method (McGonicle et al., 1990). We calculated percent colonization as the number of root sections that contained at least one AMF structure divided by the total number of sections (50 in this case).

The 2023 AMF colonization was assessed on the collected leafy spurge roots from each treatment plot following the same clearing and staining process as above, except the leafy spurge roots were cleaned in a 30% bleach solution first to reduce surface pigmentation. The Tordon plots did not have any leafy spurge in 2023 and therefore roots from these plots could not be collected for colonization analysis.

3.2.4 Statistical Analysis

We included the structural variables from the experiment in all analyses unless otherwise noted; herbicide and fertilizer treatments and year were included as fixed factorial effects, with block and plot as random effects due to repeated measures. All statistical analyses were conducted in R version 4.3.3 (R Core Team, 2024). Fertilizer errors [n=22 (Control = 1, micro = 10, N+P+Micro = 11)] were excluded from all analyses. Due to large differences in species composition and soil texture among sites, we also used soil texture as a fourth factorial fixed effect variable. For this, we grouped the Manitou and North Battleford sites together as sandy sites and the St. Denis and Grenfell sites together as loamy sites. A biplot based on non-metric multidimensional scaling ordination of the common plant species was conducted using the vegan package (Oksanen J et al., 2022) as can be seen in **Figure 3.1**.

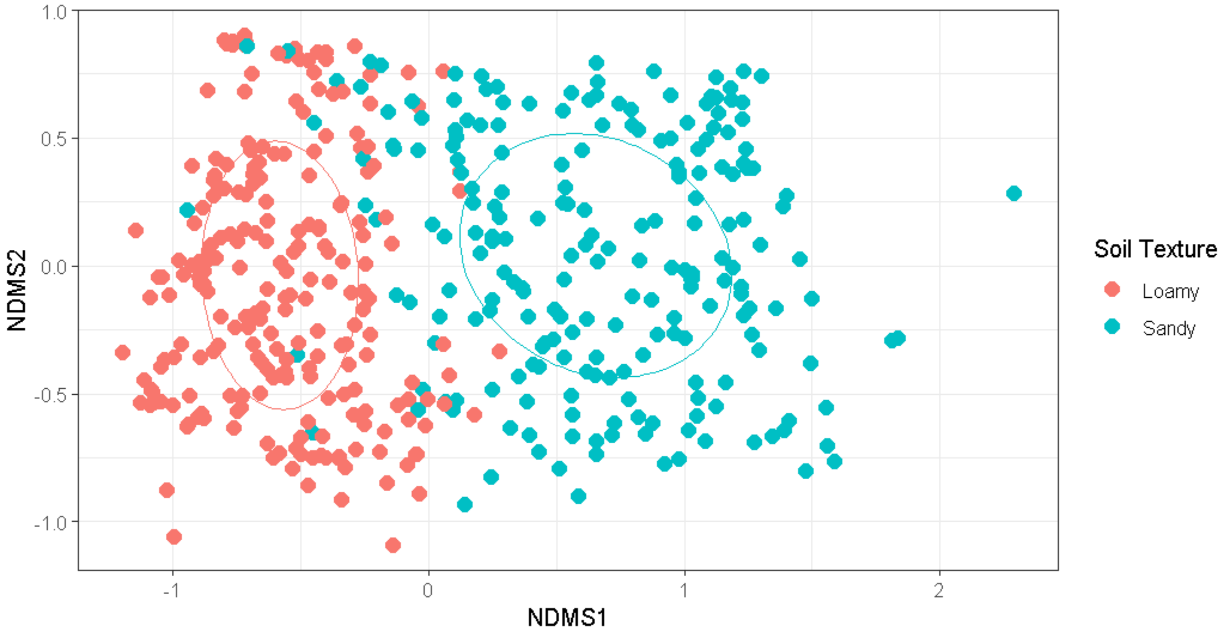


Figure 3.1: Non-metric multidimensional scaling (NMDS) of common plants by soil texture (Loamy and Sandy). Common plants were assessed as those occurring in more than 40 plots, such that infrequently occurring or rare plants were not included in the common plant population.

Leafy spurge relative cover, species richness, colonization data, and soil nutrients were assessed using a mixed effects LMER model with the lme4 package (Bates et al., 2015), and the lmerTest package (Kuznetsova et al., 2017), with graphs generated using the afex package (Singmann et al., 2024). Leafy spurge plot cover only included 2023 data; therefore, year and plot were not included in the analysis. Because the methods of colonization assessment varied between years, it was necessary to evaluate the colonization data separately by year. The herbicide Tordon was not included in the model for the 2023 colonization assessment because there were no leafy spurge plants available to collect for colonization analysis. To analyze the soil nutrient data, we chose to focus on the three soil nutrients identified in the hypotheses: N, P, and Zn. Soil nutrient data was only collected in 2022; therefore, year and plot were not included in the colonization and soil nutrient analyses. The species richness data, and 2022 colonization data were square root transformed to improve normality of the residuals. Soil nutrient data was log transformed to improve normality as was colonization data for 2023.

Given the high level of species variation among sites, we assigned plants to five different functional groups for community analysis: invasive forbs (IF) (not including leafy spurge), invasive grasses (IG), native forbs (NF), native grasses (NG), and native shrubs (NS). A permutational multivariate analysis of variance (PERMANOVA) using the vegan package (Oksanen J et al., 2022) was completed using relative cover of the plant functional groups as the response variable with block as strata using Bray-Curtis distances and 999 permutations. To further explore the herbicide-by-year interaction, we completed an indicator species analysis using the indicspecies package (Cáceres & Legendre, 2009) to identify which herbicide effects varied by year. The bar graph was created using the ggplot2 package (Wickham, 2016).

Given the complexity of the significant three-way interaction between herbicide, fertilizer, and soil texture in the plant functional groups PERMANOVA (**Table 3.13**), we chose to explore the responses of individual functional groups using mixed models for each plant functional group separately. Given the lack of a significant four-way interaction with year, we chose to focus on the 2023 data as we felt this was more representative of the overall efficacy of the treatments. Invasive forbs and grasses lacked sufficient quantities and were excluded from the independent analysis. The native forb data was log transformed, and the native shrub data was square root transformed to improve normality of the residuals. The lmerTest package

(Kuznetsova et al., 2017) was used to estimate degrees of freedom and p-values for F-tests for the mixed models with graphs generated using the afex package (Singmann et al., 2024).

3.3 Results

3.3.1 Effects of Herbicide and Fertilizer Treatments on Leafy Spurge Cover

At the plot level in 2023, both herbicides and fertilizers had significant effects, although herbicide effects depended on the soil texture (**Table 3.10**). 2,4-D provided moderate control in both soil textures, while Overdrive provided moderate control in sandy soils only (**Figure 3.2**). Navius and Tordon provided strong control in both soil textures. Micronutrient and N fertilizers reduced leafy spurge cover compared to N+P+Micro and NP, but not the Control, although it is trending lower (**Figure 3.3**).

At the quadrat level, herbicides, but not fertilizers had significant effects on leafy spurge cover, though the effects of the herbicides were dependent on the soil texture and the year of the study (**Table 3.11**). 2,4-D provided strong control of leafy spurge in the first year; however, leafy spurge relative cover recovered in the second year (**Figure 3.4**). Overdrive modestly reduced leafy spurge relative cover, but only in sandy soils. Although Navius control was limited in year one due to an application error, it provided strong leafy spurge control in year two. Tordon provided effective control in both years and soil textures (**Figure 3.4**).

Table 3.10: Results of the mixed effects model on 2023 leafy spurge cover (plot level) with herbicide, fertilizer, and soil texture as explanatory variables.

Variable	Sum Sq	Mean Sq	Df (num/den)	F value	P	Significance
Herbicide	122101	30525.2	4/279	134.561	<0.001	***
Fertilizer	2673	534.6	5/280	2.357	0.041	*
Soil Texture	2630	2630.5	1/10	11.596	0.006	**
Herbicide x Fertilizer	6414	320.7	20/279	1.414	0.114	
Herbicide x Soil Texture	13072	3267.9	4/279	14.406	<0.001	***
Fertilizer x Soil Texture	1211	242.3	5/280	1.068	0.378	
Herbicide x Fertilizer x Soil Texture	3996	199.8	20/279	0.881	0.612	

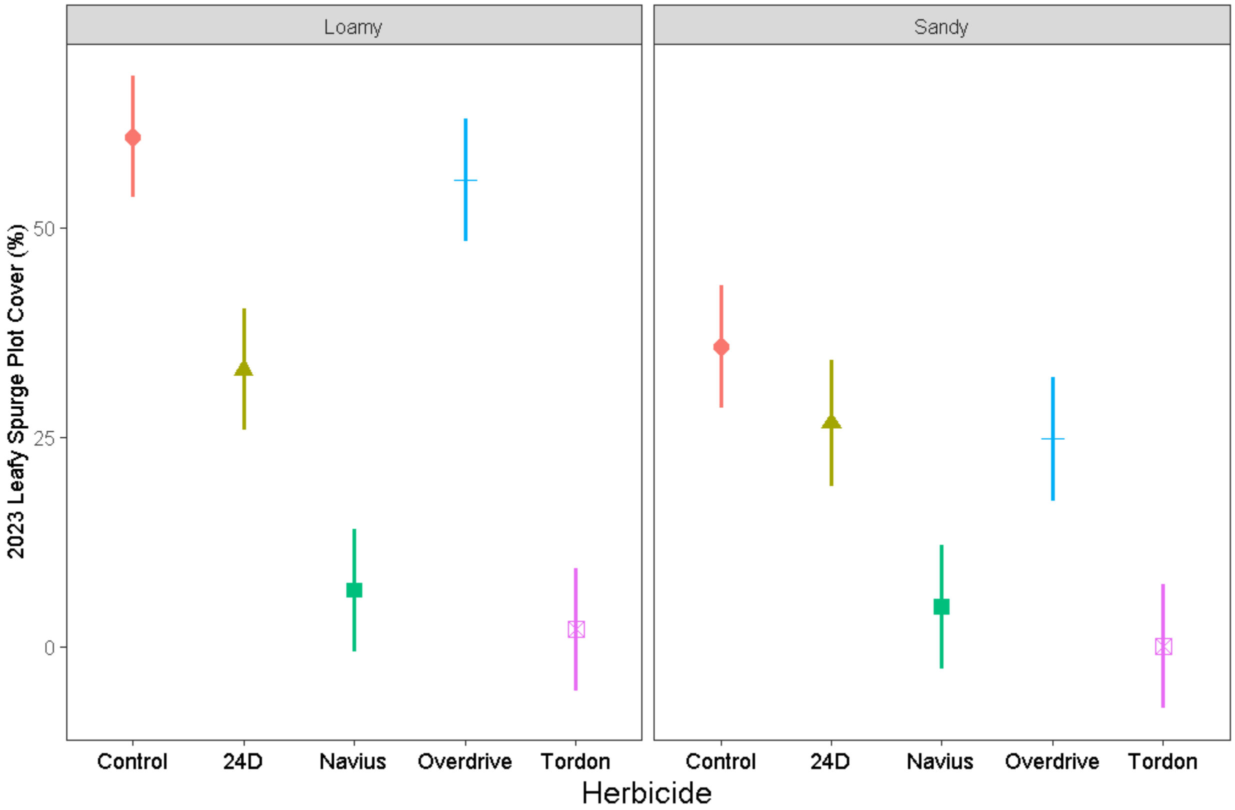


Figure 3.2: Effects of herbicide treatments on leafy spurge plot cover (%) by soil texture. Each panel is divided by soil texture. The symbols represent the estimated marginal mean leafy spurge relative cover, while the bars represent the 95% confidence interval.

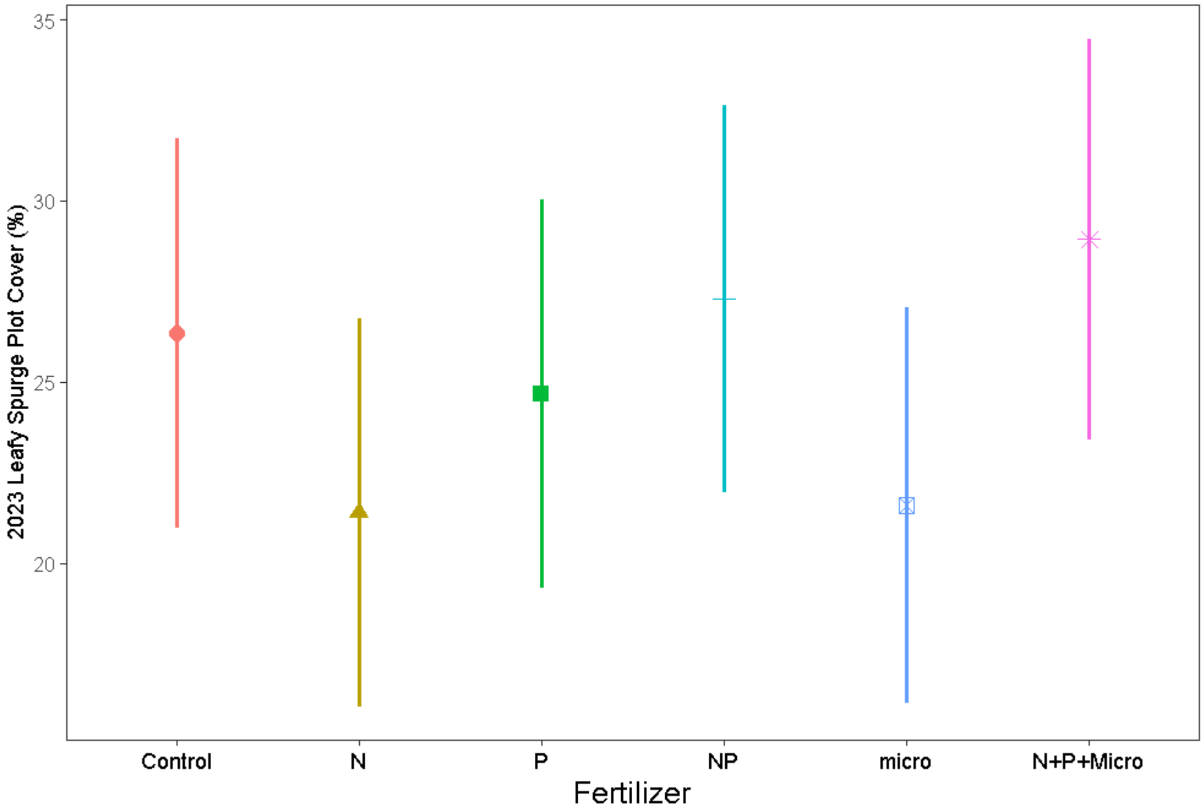


Figure 3.3: Effects of fertilizer treatments on 2023 leafy spurge plot cover (%). The symbols represent the estimated marginal mean leafy spurge relative cover, while the bars represent the 95% confidence interval.

Table 3.11: Results of the mixed effects model on leafy spurge relative cover (quadrat level) with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	**Sum Sq**	**Mean Sq**	**Df (num/den)**	**F value**	**P**	**Significance**
Herbicide	40539	10134.7	4/281	81.741	<0.001	***
Fertilizer	545	109.0	5/281	0.879	0.496	
Year	220	220.2	1/287	1.776	0.184	
Soil Texture	747	746.7	1/10	6.023	0.034	*
Herbicide x Fertilizer	1895	94.8	20/280	0.764	0.756	
Herbicide x Year	20061	5015.1	4/287	40.449	<0.001	***
Fertilizer x Year	211	42.3	5/287	0.341	0.888	
Herbicide x Soil Texture	2677	669.2	4/281	5.398	<0.001	***
Fertilizer x Soil Texture	974	194.8	5/281	1.571	0.168	
Year x Soil Texture	156	155.9	1/287	1.257	0.263	
Herbicide x Fertilizer x Year	2017	100.8	20/287	0.813	0.697	
Herbicide x Fertilizer x Soil Texture	1994	99.7	20/280	0.804	0.708	
Herbicide x Year x Soil Texture	936	234.0	4/287	1.887	0.113	
Fertilizer x Year x Soil Texture	382	76.4	5/287	0.616	0.688	
Herbicide x Fertilizer x Year x Soil Texture	1587	79.3	20/287	0.640	0.881	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

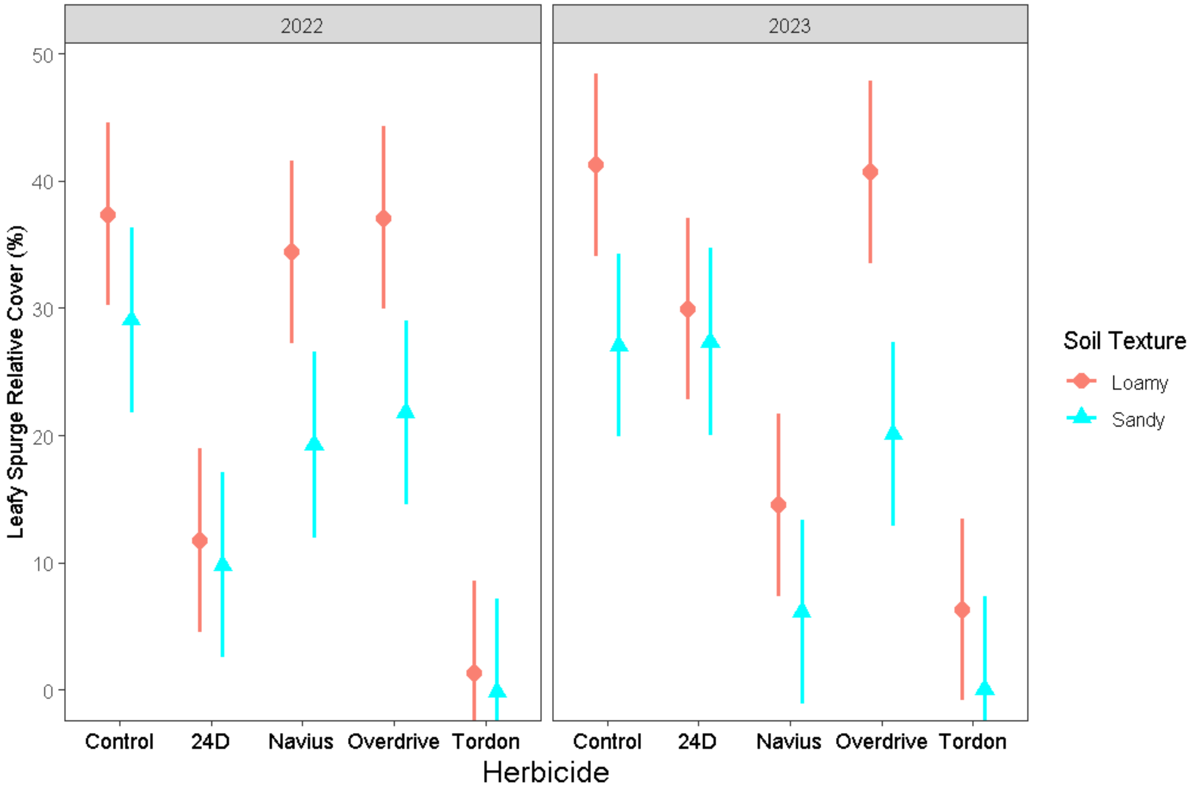


Figure 3.4: Effects of herbicide treatments on leafy spurge relative cover (%) (quadrat level) by soil texture and year. Each panel is divided by year. The symbols represent the estimated marginal mean leafy spurge relative cover, while the bars represent the 95% confidence interval.

3.3.2 Effects of Herbicide and Fertilizer Treatments on Species Richness

Species richness varied by herbicide treatment; however, the effects were dependent on both the soil texture and the year of the study (**Table 3.12**). Unsurprisingly, herbicide treatments negatively impacted species richness (**Figure 3.5**). The negative effects of the herbicide were stronger in loamy soils (**Figure 3.5**) in both years, although these effects dissipated in year two (2023) with 2,4-D and Overdrive. Tordon experienced the greatest reduction in species richness in both soil textures in both years of the study.

Table 3.12: Results of the mixed effects model on species richness with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	Sum Sq	Mean Sq	Df (num/den)	F value	P	Significance
Herbicide	18.03	4.51	4/280	42.085	<0.001	***
Fertilizer	0.47	0.09	5/280	0.878	0.496	
Year	6.29	6.29	1/289	58.760	<0.001	***
Soil Texture	0.69	0.69	1/10	6.466	0.029	*
Herbicide x Fertilizer	2.26	0.11	20/280	1.055	0.397	
Herbicide x Year	4.72	1.18	4/289	11.013	<0.001	***
Fertilizer x Year	0.40	0.08	5/289	0.756	0.582	
Herbicide x Soil Texture	1.03	0.26	4/280	2.412	0.049	*
Fertilizer x Soil Texture	0.41	0.08	5/280	0.759	0.580	
Year x Soil Texture	1.89	1.89	1/289	17.623	<0.001	***
Herbicide x Fertilizer x Year	2.04	0.10	20/289	0.950	0.523	
Herbicide x Fertilizer x Soil Texture	1.18	0.06	20/280	0.551	0.942	
Herbicide x Year x Soil Texture	0.11	0.03	4/289	0.268	0.898	
Fertilizer x Year x Soil Texture	0.73	0.15	5/289	1.354	0.242	
Herbicide x Fertilizer x Year x Soil Texture	2.01	0.10	20/289	0.937	0.540	

Significance ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05.

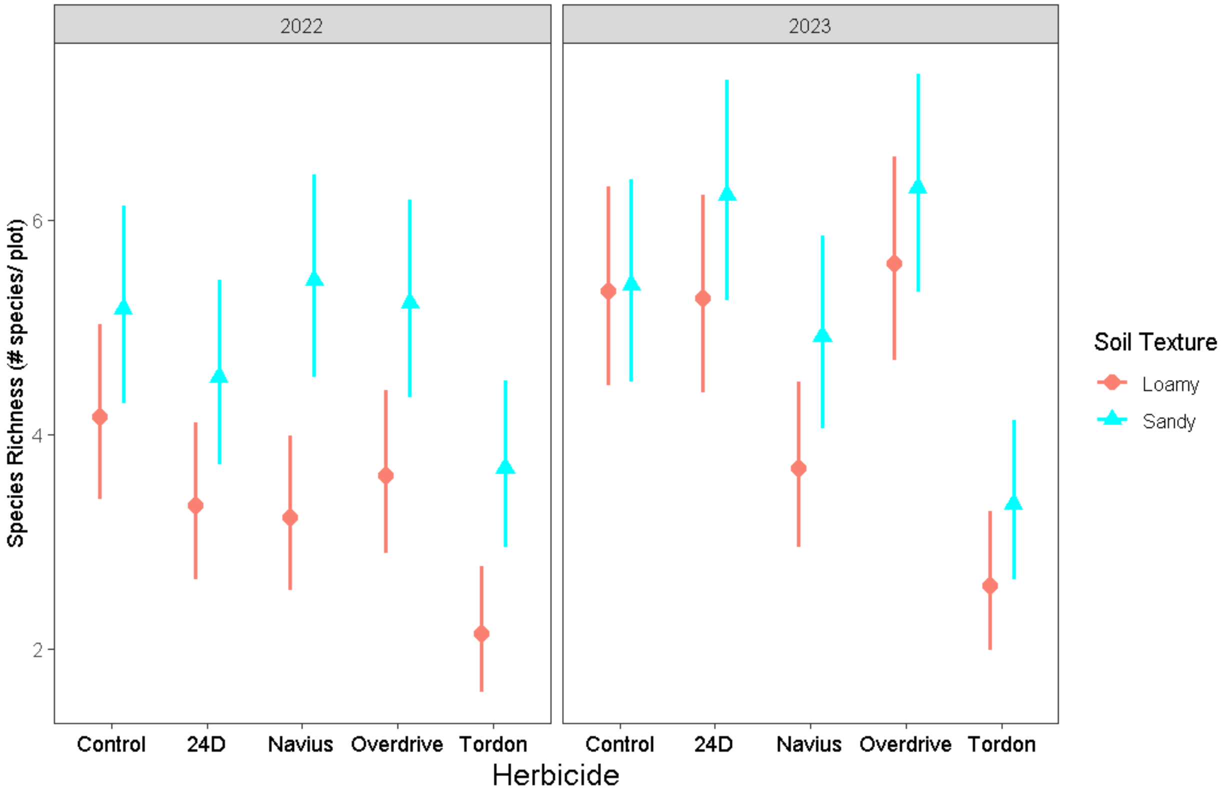


Figure 3.5: Effects of herbicide treatments on total species richness (# of species per plot) by soil texture. Each panel is divided by year. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

3.3.3 Effects of Herbicide and Fertilizer Treatments on Plant Functional Groups

The plant functional group PERMANOVA showed significant interactions between herbicide and year and a three-way interaction with herbicide, fertilizer, and soil texture, indicating complex changes in the plant community (**Table 3.13**). Native forbs were significantly affected by herbicides (**Table 3.14**), with Navius and Tordon plots showing the greatest decline (**Figure 3.6**). Native grass relative cover was increased by all herbicide treatments, except Overdrive, with Tordon plots showing the largest increase compared to the Control (**Table 3.14**, **Figure 3.7**). This effect was greatest in sandy soil, consistent with the significant herbicide-by-soil texture interaction (**Table 3.13**, **Figure 3.7**). Herbicides also had significant effects on native shrubs, but this effect was limited to sandy soils (**Table 3.14**, **Figure 3.8**). Tordon caused a loss of shrubs in both soil textures while Overdrive increased shrubs, but only in sandy soils (**Figure 3.8**). None of the more abundant plant functional groups responded to fertilizer application or its interactions with soil texture and year, however (**Table 3.14**).

Table 3.13: Results of the PERMANOVA on plant functional group relative cover with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	Df	Sum Sq	R2	F value	P	Significance
Herbicide	4	14.52	0.102	20.965	0.001	***
Fertilizer	5	0.52	0.004	0.596	0.915	
Year	1	0.29	0.002	1.691	0.058	
Soil Texture	1	10.57	0.074	61.073	0.001	***
Herbicide x Fertilizer	20	3.55	0.025	1.025	0.106	
Herbicide x Year	4	1.16	0.008	1.672	0.006	**
Fertilizer x Year	5	0.46	0.003	0.537	0.960	
Herbicide x Soil Texture	4	2.04	0.014	2.945	0.001	***
Fertilizer x Soil Texture	5	0.74	0.005	0.851	0.467	
Year x Soil Texture	1	0.20	0.001	1.148	0.191	
Herbicide x Fertilizer x Year	20	2.17	0.015	0.627	0.991	
Herbicide x Fertilizer x Soil Texture	20	4.68	0.033	1.351	0.001	***
Herbicide x Year x Soil Texture	4	0.45	0.003	0.650	0.777	
Fertilizer x Year x Soil Texture	5	0.51	0.003	0.584	0.902	
Herbicide x Fertilizer x Year x Soil Texture	20	1.74	0.012	0.504	1.000	
Residual	572	99.03	0.694			
Total	691	142.63	1.00000			

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

Table 3.14: Results of the mixed effects model on the 2023 relative cover of each plant functional group with herbicide, fertilizer, and soil texture variables. The invasive forbs and grasses lacked sufficient quantities and were excluded from the independent analysis.

	Sum Sq	Mean Sq	Df (num/den)	F value	P	Significance
Native Forbs						
Herbicide	212.24	53.06	4/278	39.68	<0.001	***
Fertilizer	5.99	1.20	5/279	0.90	0.484	
Soil Texture	1.99	1.98	1/10	1.48	0.251	
Herbicide x Fertilizer	15.21	0.76	20/278	0.57	0.932	
Herbicide x Soil Texture	8.21	2.05	4/278	1.53	0.192	
Fertilizer x Soil Texture	5.48	1.10	5/279	0.82	0.537	
Herbicide x Fertilizer x Soil Texture	12.86	0.64	20/278	0.48	0.972	
Native Grasses						
Herbicide	71959.00	17989.80	4/278	34.65	<0.001	***
Fertilizer	2657.00	531.40	5/278	1.02	0.404	
Soil Texture	812.00	812.00	1/10	1.56	0.239	
Herbicide x Fertilizer	6482.00	324.10	20/278	0.62	0.894	
Herbicide x Soil Texture	7342.00	1835.50	4/278	3.53	0.008	**
Fertilizer x Soil Texture	197.00	39.40	5/278	0.08	0.996	
Herbicide x Fertilizer x Soil Texture	12963.00	648.10	20/278	1.25	0.214	
Native Shrubs						
Herbicide	593.81	148.45	4/278	18.44	<0.001	***
Fertilizer	15.31	3.06	5/279	0.38	0.862	
Soil Texture	80.13	80.13	1/10	9.96	0.010	*
Herbicide x Fertilizer	151.63	7.58	20/278	0.94	0.534	
Herbicide x Soil Texture	122.65	30.66	4/278	3.81	0.005	**
Fertilizer x Soil Texture	13.47	2.69	5/279	0.33	0.892	
Herbicide x Fertilizer x Soil Texture	170.10	8.50	20/278	1.06	0.396	

*Significance ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05.*

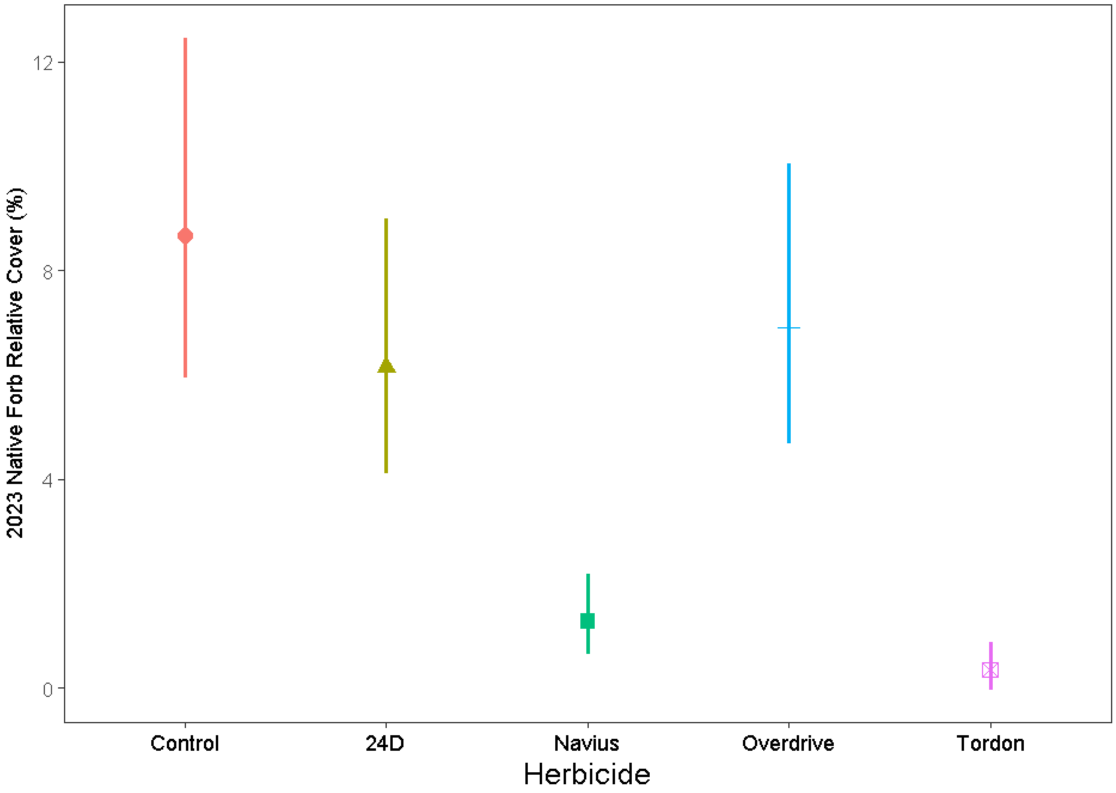


Figure 3.6: Effects of herbicide treatments on the 2023 relative cover of native forbs. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

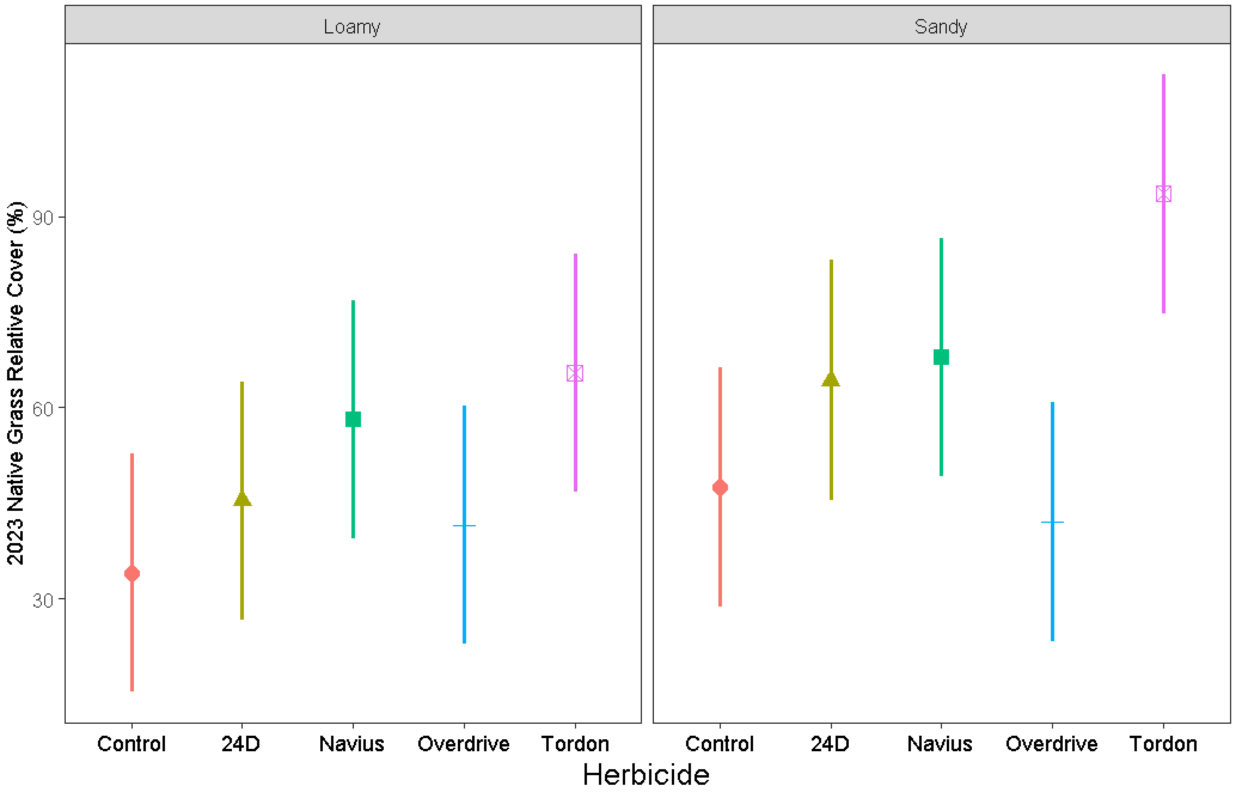


Figure 3.7: Effects of herbicide treatments on the 2023 native grass cover by soil texture. Each panel is divided by soil texture. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

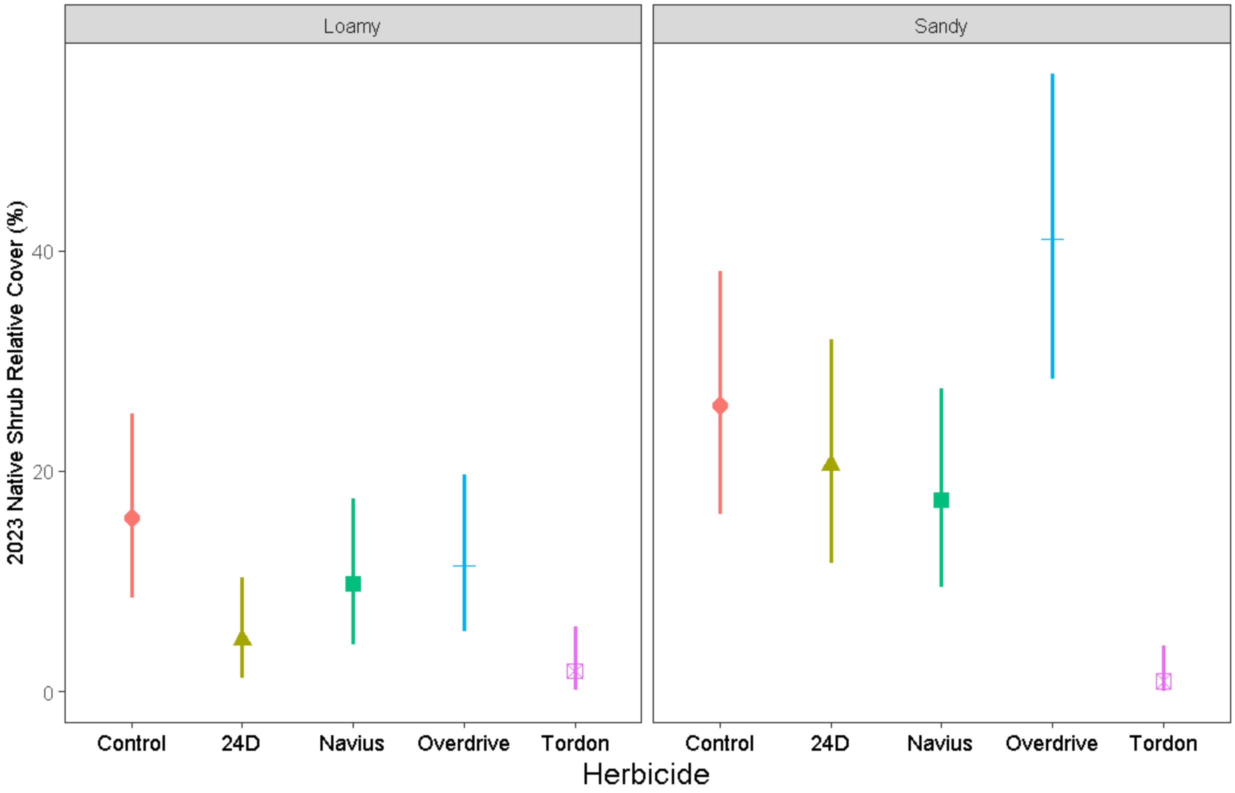


Figure 3.8: Effects of herbicide treatments on the 2023 relative cover of native shrubs by soil texture. Each panel is divided by soil texture. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

Indicator species analysis investigating the herbicide-by-year interaction (**Table 3.15**) identified that the Control and Overdrive treatments consistently had more native forbs and shrubs compared with the other herbicide treatments, whereas forbs declined in 2022 but recovered in 2023 in 2,4-D plots and Navius showed a decline in shrubs from 2022 to 2023. (**Table 3.15**, **Figure 3.9**). The 2,4-D, Navius, and Tordon treatments showed consistently more native grasses over other herbicide treatments (**Table 3.15**, **Figure 3.9**).

Table 3.15: Results of the indicator species analysis for herbicide and year on relative cover of plant functional groups. Coloured squares indicate significance with a p-value of <0.001. The invasive forbs and grasses did not significantly differ among treatments according to this analysis, so are excluded here.

Plant Functional Group Relative Cover (%)	**Herbicides**										**Significance**
	Control		**2,4-D**		**Navius**		**Overdrive**		**Tordon**		
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	
Native Forbs (NF)	🟪	🟪		🟪			🟪	🟪			0.001 ***
Native Grasses (NG)			🟩	🟩	🟩	🟩			🟩	🟩	0.001 ***
Native Shrubs (NS)	▨	▨		▨	▨	▨	▨	▨			0.001 ***

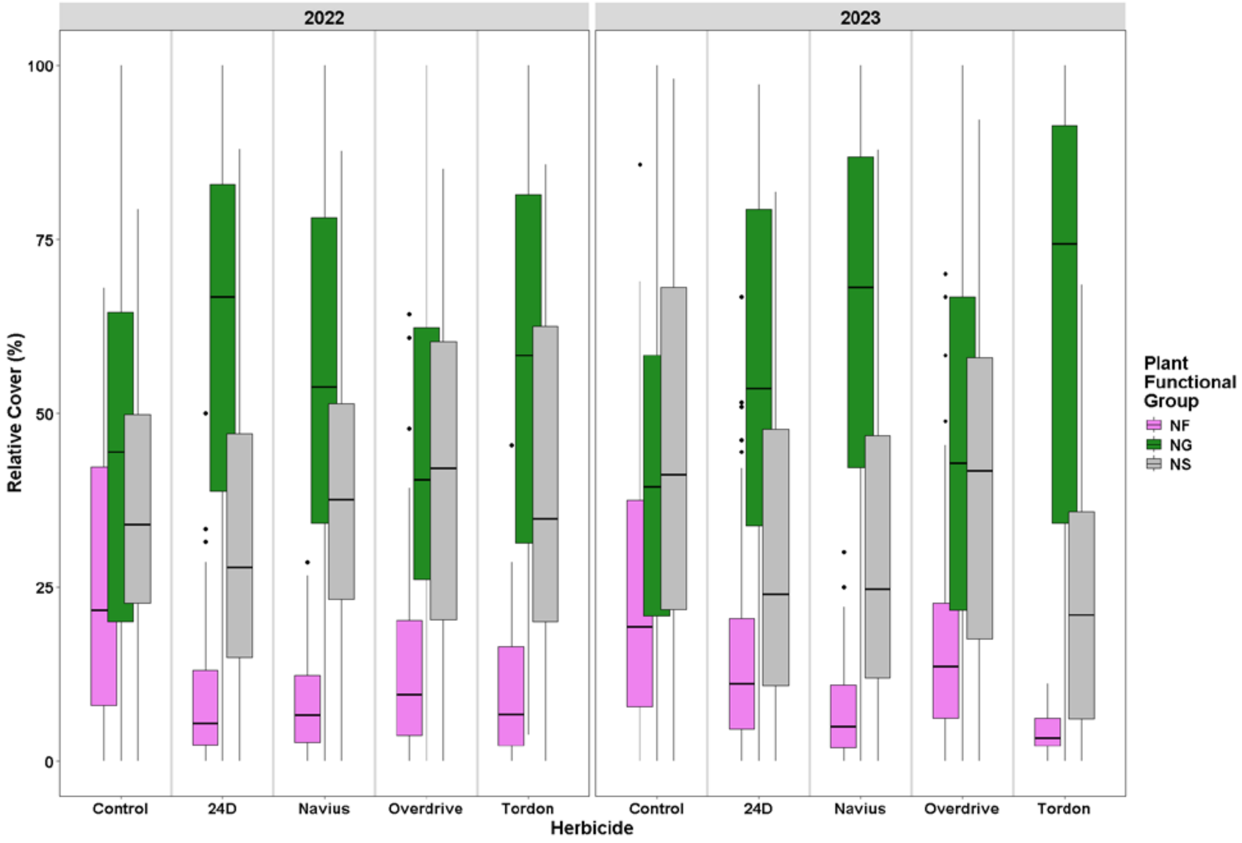


Figure 3.9: Effects of the herbicide by year interaction on plant functional groups relative cover (%). Each panel is divided by year. The horizontal line in the middle of the box represents the median value. The top of the box represents the upper quartile while the bottom of the box represents the lower quartile. The vertical lines above and below the box represent the upper and lower values of the data. Single points are outliers. NF = Native Forbs, NG = Native Grasses, NS = Native Shrubs.

3.3.4 Effects of Herbicide and Fertilizer Treatments on AMF Colonization

In 2022, fertilizer and herbicide had complex interactive effects on colonization of the phytometer, indicating complex changes in the relative abundance of AMF propagules (**Table 3.16**). Under Control conditions, phytometer colonization was enhanced by P but reduced by N+P (**Figure 3.10**). All fertilizers except the N+P+Micro treatment reduced phytometer colonization in the 2,4-D plots. The greatest decreases in phytometer colonization were with micronutrient and 2,4-D and P and Tordon. Conversely, N fertilizer treatment increased phytometer colonization, but only with Navius and Tordon.

There was a marginal fertilizer effect in 2023 (**Table 3.17**), which excluded Tordon treatments due to a lack of leafy spurge plants available to collect for colonization analysis. All fertilizer treatments increased leafy spurge colonization compared to the Control, except when N, P, and the micronutrients were applied together, which showed only a marginal increase (**Figure 3.11**).

Table 3.16: Results of the mixed effects model on 2022 phytometer percent AMF colonization data with herbicide, fertilizer, and soil texture as explanatory variables.

Variable	**Sum Sq**	**Mean Sq**	**Df (num/den)**	**F value**	**P**	**Significance**
Herbicide	0.07	0.02	4/269	0.804	0.523	
Fertilizer	0.25	0.05	5/269	2.216	0.053	
Soil Texture	0.07	0.07	1/9	2.952	0.117	
Herbicide x Fertilizer	0.87	0.04	20/268	1.942	0.010	*
Herbicide x Soil Texture	0.10	0.02	4/269	1.104	0.355	
Fertilizer x Soil Texture	0.04	0.01	5/269	0.398	0.850	
Herbicide x Fertilizer x Soil Texture	0.46	0.02	20/268	1.039	0.417	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

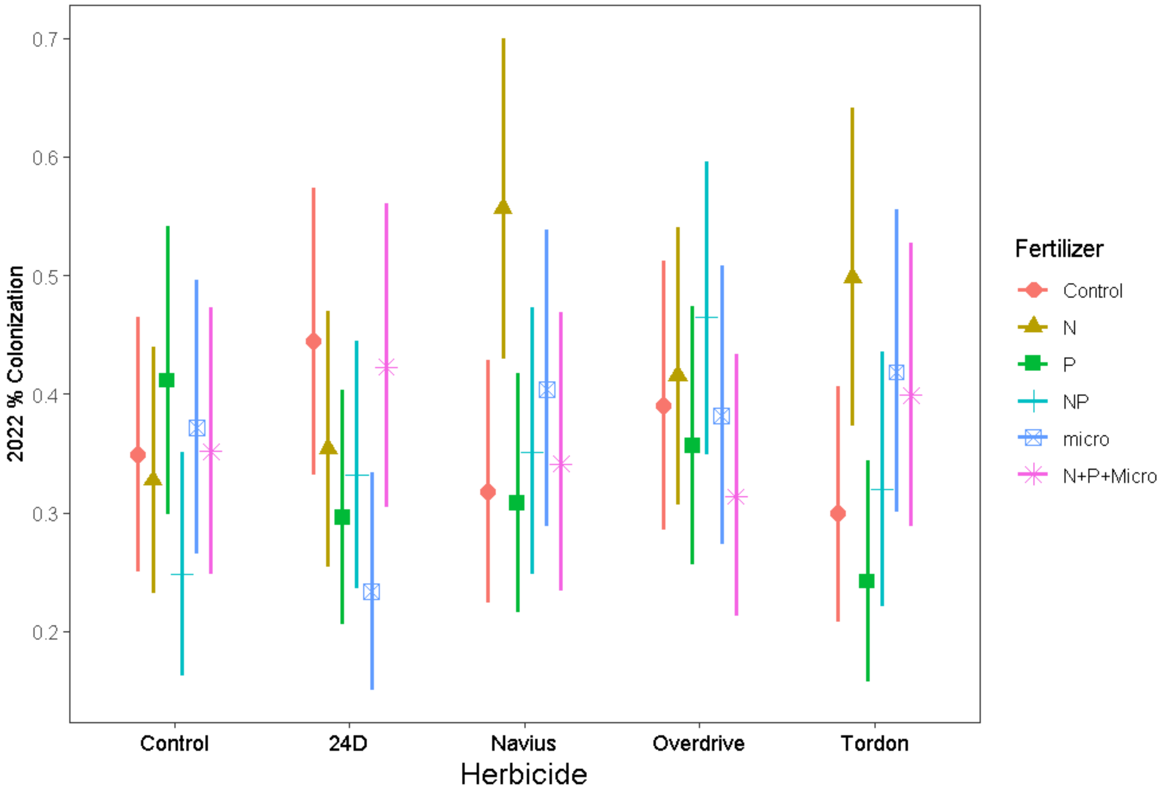


Figure 3.10: Effects of herbicide and fertilizer treatments interaction on 2022 phytometer percent AMF colonization. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

Table 3.17: Results of the mixed effects model on the 2023 leafy spurge AMF colonization data with herbicide (Tordon treatment not included), fertilizer, and soil texture as explanatory variables.

Variable	**Sum Sq**	**Mean Sq**	**Df (num/den)**	**F value**	**P**	**Significance**
Herbicide	0.17	0.06	3/153	0.960	0.413	
Fertilizer	0.65	0.13	5/152	2.181	0.059	marginal
Soil Texture	0.01	0.01	1/10	0.186	0.675	
Herbicide x Fertilizer	1.10	0.07	15/152	1.220	0.262	
Herbicide x Soil Texture	0.13	0.04	3/153	0.743	0.528	
Fertilizer x Soil Texture	0.06	0.01	5/152	0.205	0.960	
Herbicide x Fertilizer x Soil Texture	1.03	0.07	15/152	1.144	0.322	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

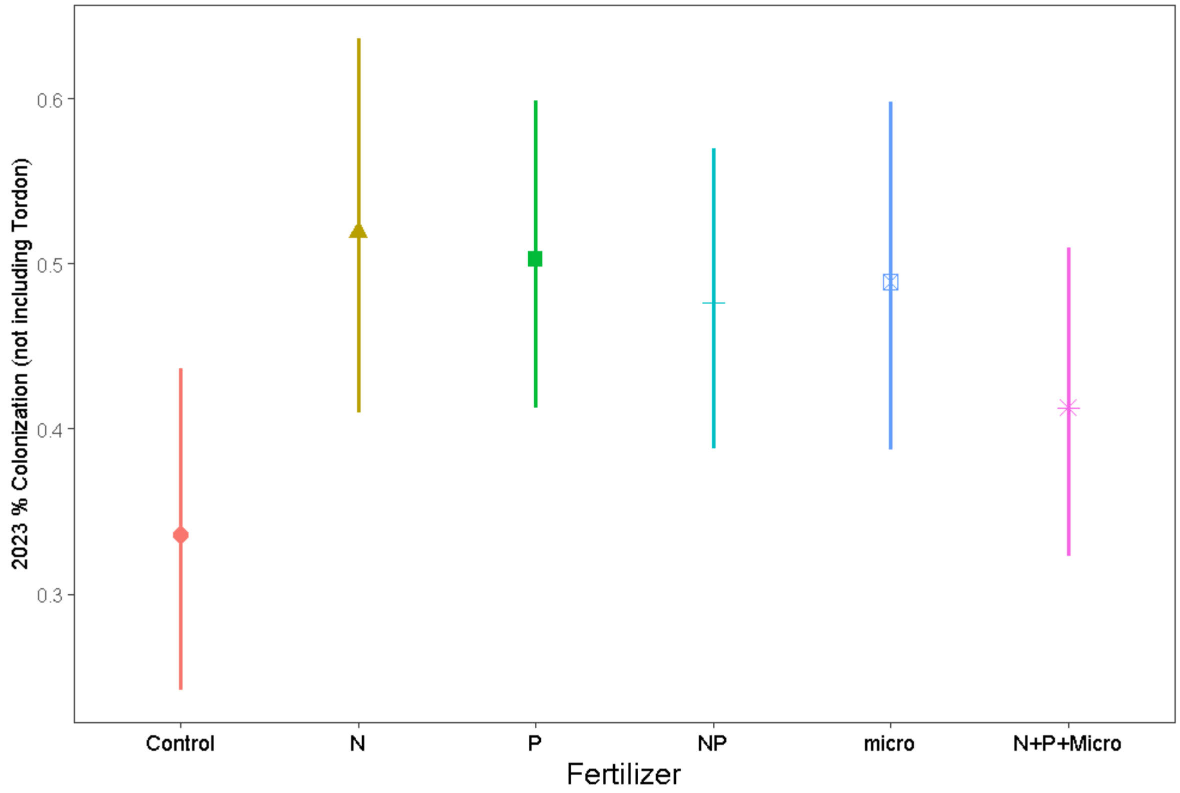


Figure 3.11: Effects of fertilizer treatments on 2023 leafy spurge AMF percent colonization. Tordon was excluded from the analysis because there were no leafy spurge plants in these plots to collect for colonization analysis. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

3.3.5 Effects of Herbicide and Fertilizer Treatments on Soil Nutrients

Treatment effects on total N, P, and Zn were complex (**Table 3.18**). Each of 2,4-D, Navius, and Tordon significantly increased N (**Figure 3.12**) and Zn (**Figure 3.15**) relative to the Control. As expected, all N fertilizer treatments (N, N+P, and N+P+Micro) significantly increased N, especially the lone N treatment in loamy soil (**Figure 3.13**). Sandy soils had greater P availability as did plots where P fertilizer was applied, however, the effect of P addition was minimal in loamy sites when also applied with N, but not with N and micronutrients (**Figure 3.14**). The micronutrient and N fertilizer treatments (N, N+P, and N+P+Micro) all increased Zn, with the N+P+Micro treatment showing enhanced levels of Zn (**Figure 3.16**).

Table 3.18: Results of the mixed effects model for Total N, P, and Zn soil nutrients with herbicide, fertilizer, and soil texture as explanatory variables.

	Sum Sq	**Mean Sq**	**Df (num/den)**	**F value**	**P**	**Significance**
Total N						
Herbicide	68.30	17.07	4/280	18.635	<0.001	***
Fertilizer	260.26	52.05	5/281	56.813	<0.001	***
Soil Texture	0.18	0.18	1/10	0.200	0.664	
Herbicide x Fertilizer	15.78	0.79	20/280	0.861	0.637	
Herbicide x Soil Texture	1.55	0.39	4/280	0.423	0.792	
Fertilizer x Soil Texture	16.93	3.38	5/281	3.695	0.003	**
Herbicide x Fertilizer x Soil Texture	15.16	0.76	20/280	0.827	0.680	
P						
Herbicide	2.60	0.65	4/280	1.203	0.309	
Fertilizer	136.93	27.38	5/281	50.691	<0.001	***
Soil Texture	10.20	10.20	1/10	18.881	0.001	**
Herbicide x Fertilizer	11.08	0.55	20/280	1.025	0.432	
Herbicide x Soil Texture	3.63	0.91	4/280	1.679	0.155	
Fertilizer x Soil Texture	12.41	2.48	5/281	4.594	<0.001	***
Herbicide x Fertilizer x Soil Texture	11.96	0.60	20/280	1.107	0.341	
Zn						
Herbicide	1.07	0.27	4/280	2.759	0.028	*
Fertilizer	6.18	1.23	5/280	12.762	<0.001	***
Soil Texture	7.00e-4	7.00e-4	1/10	0.007	0.934	
Herbicide x Fertilizer	2.35	0.12	20/280	1.213	0.242	
Herbicide x Soil Texture	0.15	0.04	4/280	0.381	0.822	
Fertilizer x Soil Texture	0.52	0.10	5/280	1.083	0.370	
Herbicide x Fertilizer x Soil Texture	1.24	0.06	20/280	0.641	0.880	

*Significance codes: '***' 0.001 '**' 0.01 '*' 0.05.*

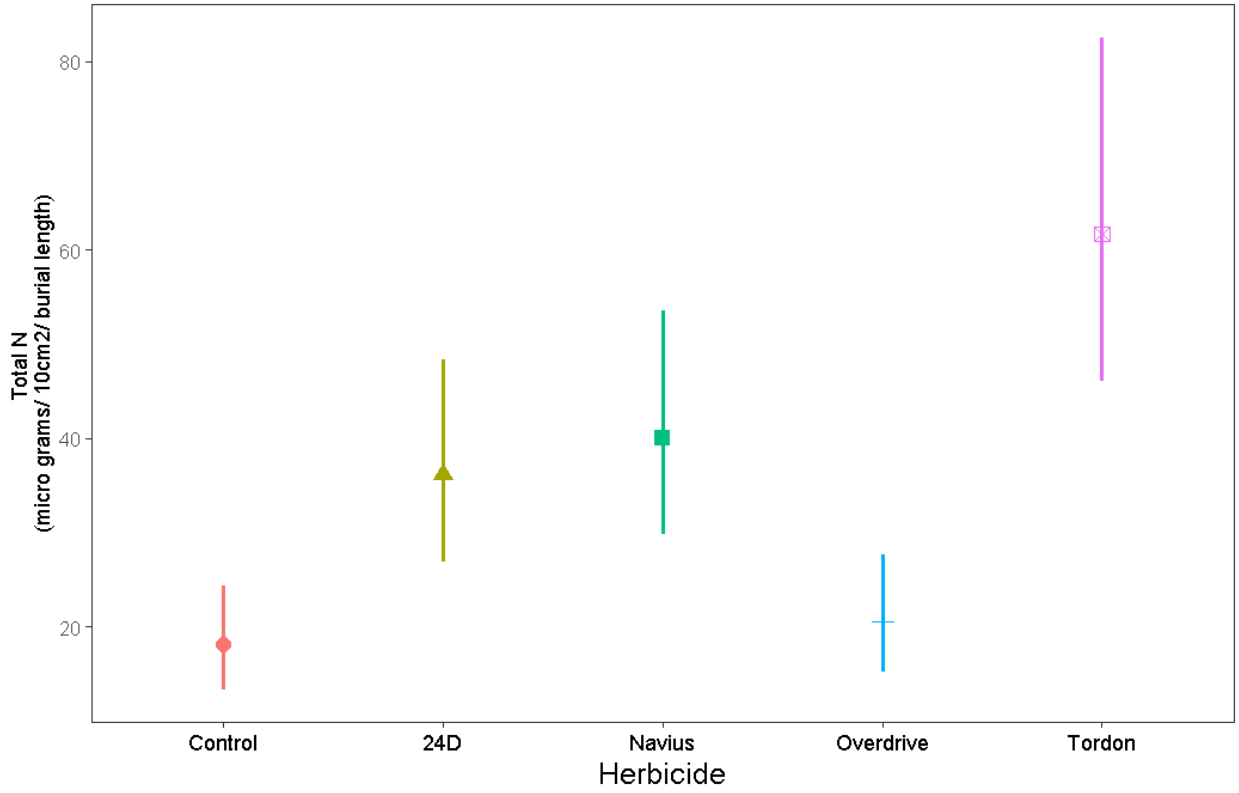


Figure 3.12: Effects of herbicide treatments on Total N soil nutrient (micro grams/ 10 cm^2/ burial length) in 2022. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

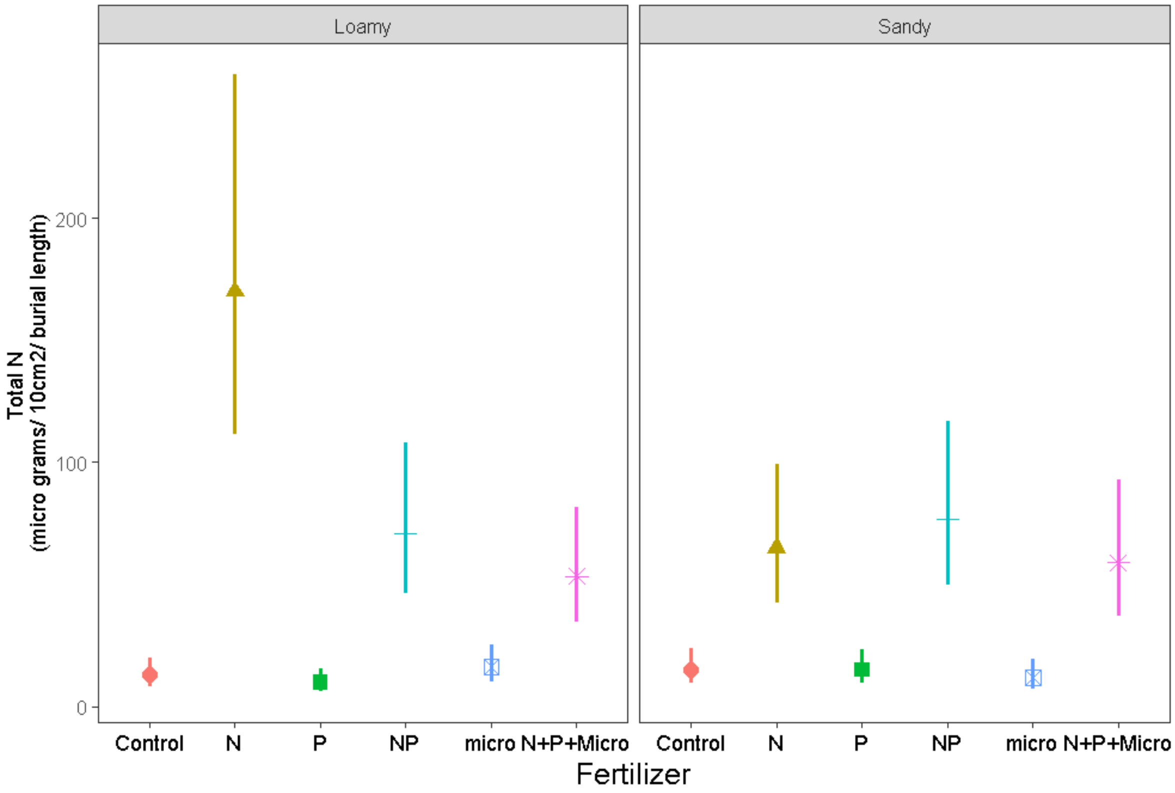


Figure 3.13: Effects of fertilizer treatments by soil texture on Total N soil nutrient (micro grams/ 10cm^2/ burial length) in 2022. Each panel is divided by soil texture. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

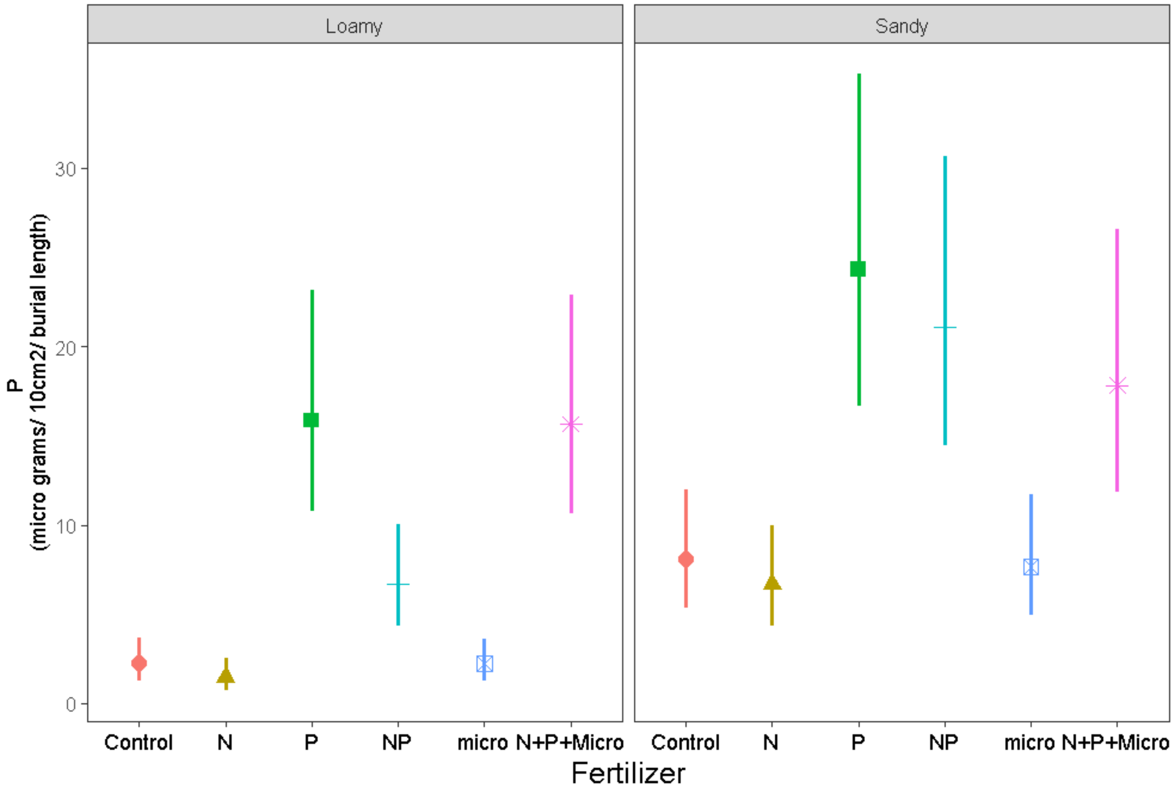


Figure 3.14: Effects of fertilizer treatments by soil texture on P soil nutrient (micro grams/10 cm^2/burial length) in 2022. Each panel is divided by soil texture. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

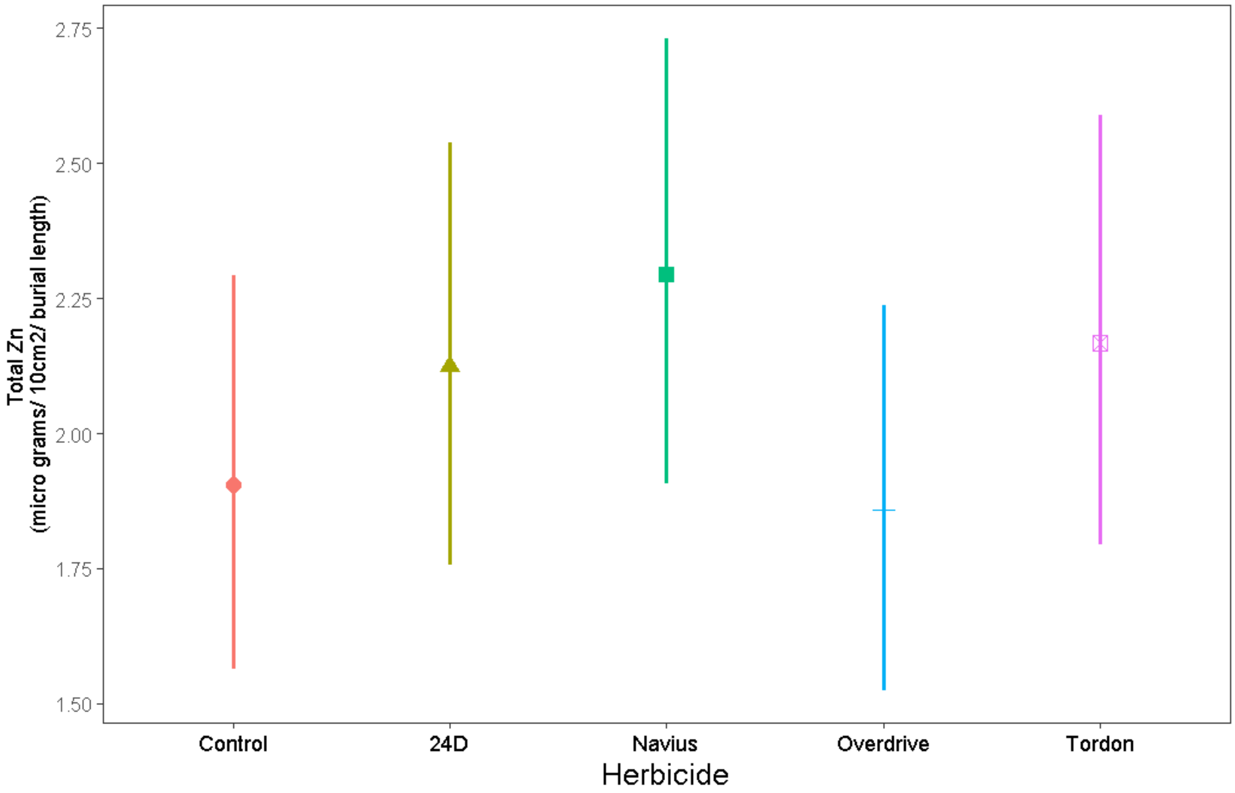


Figure 3.15: Effects of herbicide treatments on Zn soil nutrient (micro grams/10 cm^2/burial length) in 2022. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

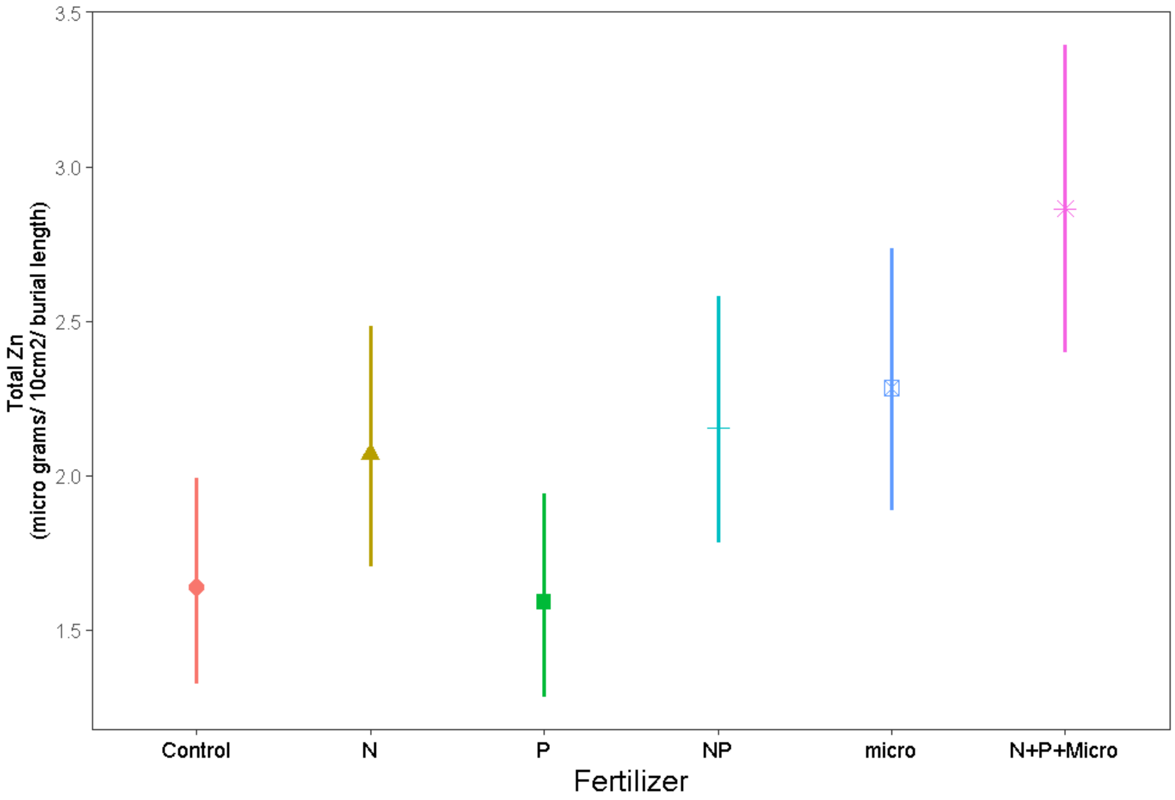


Figure 3.16: Effects of fertilizer treatments on Zn soil nutrient (micro grams/10 cm^2/burial length) in 2022. The symbols represent the estimated marginal mean, while the bars represent the 95% confidence interval.

3.4 Discussion

Herbicides more so than fertilizers caused changes in the plant community. Herbicides decreased leafy spurge cover, reduced species richness, altered the relative abundance of the different plant functional groups, impacted AMF colonization, and modified soil nutrients, although these effects were typically herbicide specific. Fertilizers affected leafy spurge cover at the plot level, plant functional group composition, AMF colonization, and soil nutrient availability. Interactive effects between the herbicides and fertilizers were only noted with the plant functional groups and the 2022 AMF colonization.

The hypothesis that fertilizer treatments would enhance herbicide efficacy resulting in reduced leafy spurge cover found only weak support as fertilizer treatments only affected leafy spurge cover at the plot level, but not the quadrat level. It is unclear why fertilizer effects were only observed at the plot level, but within plot heterogeneity may have obscured any effect of the fertilizer. Further, we found no interactive effects of herbicide and fertilizer with leafy spurge cover, suggesting that any fertilizer effect may be independent of herbicide application. These results indicate that fertilizer effects are not strong at least initially, although the complex effects that fertilizers had on AMF may result in changes in leafy spurge abundance over time. Weak fertilizer effects may have been caused by a calculation error which resulted in less fertilizer than desired being applied in year one (2022). While the fertilizer application amount was corrected for year two (2023), it is possible that a threshold was not achieved to sufficiently affect mycorrhizal benefits for leafy spurge. It is also possible that the nutrients will have a lagged effect or were not available at the right time as the region was undergoing significant drought (Schärer et al., 2023). Other studies have found limited efficacy of fertilizer for invasive weed control. Jacobs and Sheley (1999) examined the effects of 2,4-D and N fertilizer and found the two did not interact to affect spotted knapweed (*Centaurea maculosa* Lam.) density or biomass; although 2,4-D and N fertilizer did interact to increase grass density, which they hypothesized to enhance long-term control (Jacobs & Sheley, 1999). Similarly, Sheley and Jacobs (1997) found no effect of N fertilizer on spotted knapweed when applied with picloram over a two year time period.

Among the herbicides, both Navius and Tordon provided multi-year control, likely due to their persistence in the soil (Helling, 2005). 2,4-D provided effective control of leafy spurge relative cover in 2022; however, the effect was only short-term due to 2,4-D's limited residual effects resulting in the recovery of leafy spurge relative cover in 2023. Overdrive was not effective in loamy soils in either year, with moderate effectiveness observed in sandy soils, likely because Overdrive only offers top growth suppression of leafy spurge (BASF Canada Inc., n.d.-b). Consequently, residual Group 4 herbicides remain the most effective means of controlling leafy spurge (Lajeunesse et al., 1997; Lym & Messersmith, 1985).

Unsurprisingly, herbicides reduced species richness, consistent with several other studies (Carter & Lym, 2018; Greet et al., 2016; Lugar et al., 2023). In year one, 2,4-D reduced species richness compared to the unsprayed plots, however species richness recovered in year two and slightly exceeded the species richness of the unsprayed plots. This recovery and slight increase in species richness in the 2,4-D plots may be attributed to the non-residual effects of the herbicide. Additionally, although the 2,4-D plots saw leafy spurge recovery in year two, leafy spurge cover remained less than the unsprayed plots and thus niche areas remained open to allow for greater forb recovery. Our results showed that Navius and Tordon also reduced forb cover and decreased species richness, with Tordon reducing species richness more than Navius. These results are supported by Greet et al. (2016) who similarly found that both AMCP + MSM (Navius) and picloram (Tordon) reduced forb cover and decreased species richness. Herbicides are known to have detrimental effects on non-target plants and can undermine conservation management goals in ecological restoration. Skurski et al. (2013) found native forb recovery had not returned to pre-treatment levels after three years suggesting recovery may be site specific or require a longer time frame. However, Lugar et al. (2023) found that herbicide impact (picloram and aminopyralid in their study) on species richness recovered to pre-treatment levels by year six. This suggests that even though species richness was negatively impacted by Navius and Tordon in our study, over time these impacts may recover, but that the recovery may be site specific. Leafy spurge, however, will also recover and it is unclear whether repeated use of herbicides to control leafy spurge will have longer term impacts on the recovery potential of the native plant community.

As expected, herbicides had strong effects on the relative abundance of individual plant functional groups, as all herbicides were broadleaf (forbs and shrubs) specific. Overdrive was the lone exception here as it had limited effects on most vegetation and increased shrub cover in sandy soils. As a stand-alone product, Overdrive is not effective on creeping perennial species (Enloe & Kniss, 2009), and as such is typically recommended as a tank mix with picloram-based products, like Tordon (Almquist et al., 2015). Thus, Overdrive may lack effectiveness for perennial plant control when used on its own. The increased shrub cover experienced with Overdrive may be explained by hormesis, which is the stimulatory effect of a subtoxic dose of a herbicide (Duke & Dayan, 2011). Low doses of glyphosate, for example, are known to stimulate plant growth, especially in woody plants (Duke & Dayan, 2011). The mechanism for glyphosate hormesis may be explained by the inhibition of lignin synthesis resulting in increased cell wall plasticity and a prolonged plant growth period (Duke & Dayan, 2011; Jalal et al., 2021). Overdrive is not labeled for control of shrubs and when combined with the initial surfactant application error, it is possible that the dose used to control the non-woody broadleaf species was sublethal for the shrub species in our plots resulting in stimulated growth by the herbicide. Further studies on dose-response are needed to better understand and estimate the dose range that promotes a homeostatic response from different chemical groups (Jalal et al., 2021).

Broadleaf specific herbicides often facilitate the recruitment of grasses (Lekberg et al., 2017; Ortega & Pearson, 2010; Skurski et al., 2013). We hypothesized that the reduced presence of leafy spurge following the selective herbicide treatments decreased competition and allowed for an increase in grass cover, with invasive grass response dependent on initial abundance. Other studies have found that reduced forb competition following broadleaf herbicides provides space for grass species to establish and shifted the plant community toward a grass-dominated landscape (Thilmony & Lym, 2017). Although herbicides like picloram can benefit native grasses (Ortega and Pearson (2010) and Skurski et al. (2013)), they can also have a strong positive effect on invasive grass (Ortega and Pearson (2010), Endress et al. (2012), and Skurski et al. (2013)). Anecdotally, we observed that the reduced cover of leafy spurge gave space for invasive grasses such as smooth bromegrass (*Bromus inermus* Leyss) and Kentucky bluegrass (*Poa pratensis* L.) to increase; however, invasive grasses were too rare at our sites to test this hypothesis. Nonetheless, the application of broadleaf selective herbicides at sites invaded with invasive grasses may pose additional harm as the herbicide can facilitate further invasion.

The observed differences in herbicide activity in differing soil textures may be explained by the complex interactions between the chemicals and the soil environment (Helling, 2005). Soil texture is strongly linked to variations in soil properties including particle size, soil organic matter, and clay content (McGinley et al., 2022), which are also linked to soil water holding capacity. Additionally, these soil factors are also critical in influencing the behaviour of herbicides in the soil (Sarkar et al., 2020). Clay content in the soil holds a negative charge with a large specific surface area which allows for greater binding of herbicides compared with course-textured or sandy soils (Sarkar et al., 2020). Soil organic matter (SOM), with an even higher cation exchange capacity (CEC) than clay, is one of the strongest determinants of herbicide mobility and transformation in the soil (Sarkar et al., 2020). SOM can form complexes with herbicides causing them to become immobile (Sarkar et al., 2020). Thus, the higher the clay and SOM content in the soil, the more likely that the herbicide will bind and become restricted. In our study, herbicide efficacy was reduced in loamy soils compared to sandy soils, likely because the loamy soils were comprised of greater organic matter, clay content, and higher soil moisture which reduces the phytotoxicity of herbicides (Schoenau et al., 2005). Additionally, sandy textured soils have lower water holding capacity resulting in lower microbial activity (Schoenau et al., 2005). Xia et al. (2020) report that soil texture is the second most important factor after soil pH in shaping the soil microbial community. Thus, the lower microbial activity in sandy soils may contribute to reduced breakdown of the herbicide resulting in longer herbicide persistence and more effective weed control.

Fertilizers and herbicides had complex effects on mycorrhizal colonization. Herbicides indirectly impact AMF by killing its host plant (Hage-Ahmed et al., 2019; Lekberg & Gibbons, 2016). Additionally, the shift in the plant community toward grasses in response to the herbicides also impacts the AMF community as forbs are generally better hosts than grasses (Reinhart et al., 2012). Thus, a shift in the plant community toward grasses has the potential to reduce AMF abundance (Lekberg et al., 2017). Direct impacts from herbicides can decrease the population of soil microbes, including AMF, depending on the phytotoxic nature of the herbicide (type and concentration), the microbial species, and the environmental conditions including soil texture (Ogidi & Akpan, 2023). Mycorrhizal colonization is also impacted by fertilizers. Inorganic N and P fertilizers, such as we applied in our study, can reduce plant mycorrhizal dependency and thus colonization (Alori et al., 2020; Ogidi & Akpan, 2023). Nitrogen fertilizers modify the pH of the soil which in turn impacts the composition of the soil microbial community as some AMF species are more sensitive to these altered conditions (Hassan et al., 2013; Ogidi & Akpan, 2023). However, in our study, the lone application of N enhanced 2022 colonization in the Navius and Tordon plots, while N fertilizer combinations (N+P and N+P+Micro) did not. Fornara et al. (2020) report that a single application of inorganic P fertilizer to a grassland can cause significant reductions in AMF colonization rates, but our results showed no significant effect. However, the P may have reduced the ability of the AMF to respond to the N treatments. These conflicting findings with nutrient additions suggest that results may vary due to different plant species, soil types, base levels of soil N, the form of N fertilizer used, or the dose applied (Hassan et al., 2013). Albornoz et al. (2024) found that nutrient enrichment impacts AMF communities and potentially their role in plant invasion, although this can vary depending on site specific conditions and the nutrients added. The added effects of herbicides only confounds the matter further. Afata et al. (2024) examined the effects of glyphosate and soil microbial activity with micronutrients and found glyphosate forms complexes with micronutrients which inhibits microbial soil functions and leads to a decrease in microbial populations. In our 2,4-D treated plots, micronutrient fertilizer treatment decreased 2022 AMF colonization. Thus it’s possible that the 2,4-D, with limited soil residual, acted in a similar way to the glyphosate in the Afata et al.

(2024) study, leading to reduced AMF populations or in someway prevented AMF colonization in these plots. To tease apart these complex effects, more directed study would be required.

The variation between our two colonization assessment methods may have contributed to differences in the results between years as each method assesses a different aspect of the AMF life history. By looking at colonization of a phytometer over a short time period, our 2022 assessment measured the production of propagules while the 2023 assessment measured vegetative structures on a leafy spurge host. Each method reflects a different aspect of AMF life history, which may explain the difference in results. Additionally, each method could also be measuring the abundance of different types of AMF, as AMF species differ in their allocation to intraradical hyphae and spore production, and weedy species that produce more spores can be more common after disturbance, such as herbicide use (Helgason et al., 2007; Powell et al., 2009). Further, if leafy spurge is specific in its AMF partners, the plant may be selecting for or against AMF species exhibiting these traits.

3.5 Conclusion

As expected, Navius and Tordon were effective for multi-year control of leafy spurge. However, these herbicides were also associated with decreased species richness and native forb loss, resulting in a shift in the plant community toward grasses. Native grasses increased with Navius application in loamy soils and with Tordon in both loamy and sandy soils, although either may still cause increases in invasive grasses. Tordon was also associated with a greater loss of native shrubs and a greater increase in Total N compared to other herbicides in the study. Thus, land management goals need to be considered with the impacts of herbicide used to control invasive plant species, including impacts on species richness and the potential for invasive grass increases, especially in areas where there is an established population.

Fertilizers boosted soil nutrient concentrations, along with enhancing leafy spurge control at the plot level with micronutrient and N, however this fertilizer effect was not realized at the quadrat level in the first two years of this study. As hypothesized, micronutrient and P fertilizers decreased initial AMF infectivity, but only when applied with 2,4-D and Tordon respectively, which also decreased the abundance of the host plant leafy spurge. Conversely, N fertilizer increased colonization when applied with residual herbicides, Navius and Tordon. All fertilizer treatments increased 2023 AMF colonization with N and micronutrient decreasing leafy spurge cover at the plot level, suggesting that the N and micronutrient fertilizers are enhancing AMF colonization and parasitism in leafy spurge. Thus, it may be possible to artificially manage AMF colonization, increase AMF parasitism, and ultimately enhance control of leafy spurge by combining herbicides and fertilizers, though continued research is required.

Chapter 4: Herbicide and Fertilizer Treatment Effects on Forage Quality and Quantity

4.1 Introduction

Grasslands are foundational to domestic livestock production (Cason & Sleugh, 2023) where forage quality and quantity are the primary ecosystem resources of importance to producers. Both quality and quantity of pasture grasses directly influence livestock gains and ranch profitability (Barr, 2023). Invasive plants compete with forage grasses for resources and can interfere with grazing as some invasive species are unpalatable or toxic to livestock (Cason & Sleugh, 2023). Herbivores, and more specifically cattle, are selective foragers that choose more palatable species and avoid areas with unpalatable species, commonly invasive plants (Cason & Sleugh, 2023; Sanaei et al., 2019). Consequently, invasive plants can reduce forage yield, degrade forage quality, decrease carrying capacity, reduce livestock performance, and diminish land values (Cason & Sleugh, 2023; DiTomaso, 2000; Sather, Kallenbach, et al., 2013; Sleugh et al., 2023).

Leafy spurge [*Euphorbia virgata* Wald & Kit (previously known as *Euphorbia esula* L.) see Flora of North America (Riina et al., 2020)] is an aggressive invasive forb with a significant presence across the Northern Great Plains (Best et al., 1980). Because of its vigorous clonal character and strong competitive abilities, leafy spurge is extremely difficult to control (Gucker, 2010). It also reduces forage quality and quantity, resulting in increased livestock production costs (DiTomaso, 2000). Additionally, leafy spurge is unpalatable to most livestock because of the milky white sap it produces. This sap contains the toxic compound ingenol, which can irritate the skin causing blisters and can aggravate the digestive tracts of animals resulting in diarrhea and weakness (St. John & Tilley, 2014). Hein and Miller (1992) investigated the influence of leafy spurge cover on forage utilization by cattle and found a strong negative relationship. At 10% leafy spurge cover, forage utilization dropped to 45% and continued to decrease rapidly as leafy spurge cover increased (Hein & Miller, 1992). Thus, cattle will avoid grazing areas even lightly infested with leafy spurge (DiTomaso, 2000).

Controlling invasive plant species can preserve pasture health and prevent accidental plant poisonings that reduce animal performance (Barr, 2023). Selective herbicides are a vegetation management tool designed to specifically target broadleaf weeds without harming grasses. These are often the tool of choice in native pastures and rangelands, where grasses show rapid improvement resulting in increased carrying capacity (Sleugh et al., 2023). Results of a weed control study in Brazil showed excellent correlation between weed control and biomass production, where herbicide treated plots showed higher biomass production compared to untreated plots, leading to improved land productivity, and increased carrying capacity (Cason & Sleugh, 2023). Sheley et al. (2000) found that herbicide treatments increased grass biomass from 173 kg/ha in nontreated controls to 1309 kg/ha with picloram (the active ingredient in Tordon). Grazing distribution can also improve following herbicide application as Sather, Kallenbach, et al. (2013) found cattle distribution increased 1.3 to 5 times in herbicide treated areas compared to nontreated pasture areas. Additionally, some herbicides have demonstrated increased forage quality by reducing ADF and NDF of harvested forage (Israel et al., 2016), suggesting strong overall benefits for livestock production.

Fertilizers are often recommended to augment weed control in pasture and can be beneficial for pasture forages by improving forage production (Miller, 2016). The effects of fertilizer on forage quality, however, are less consistent. Nitrogen fertilization of a pasture in Turkey demonstrated significant increases in yield response and crude protein content while also decreasing NDF and ADF values (Balabanli et al., 2010). Similarly, in Canadian tame pastures, herbicides combined with fertilizers increased forage yield and quality (Bork et al., 2007). However, other studies have found that, despite increases in forage quantity, N fertilizer had minimal effect on forage quality (Bailey et al., 2024; Dindová et al., 2019). Further, most of these studies have focused on nitrogen fertilizer as this is the most common limiting nutrient. The effects of other fertilizer types on weed control, forage production, and forage quality are poorly studied. Thus, further study of fertilizer use to improve invasive weed control is needed to understand how it may impact forage quality and quantity.

The goal of this study is to determine the optimal herbicide and fertilizer combination that increases grass productivity while effectively controlling leafy spurge. We hypothesize that nitrogen (N), phosphorus (P), and micronutrient fertilizers, particularly zinc (Zn), will increase grass productivity and improve forage quality while minimizing leafy spurge abundance.

4.2 Materials and Methods

4.2.1 Study Sites

We selected four sites for the project: St. Denis, Grenfell, North Battleford, and the Manitou Community Pasture located south of Marsden, SK (Please see **Chapter 3: 3.2.1 Study Sites** for ecological information and climatic details for each study site. Additionally, **Table 3.1** provides location information for each research block). These sites were all native prairie grassland, had sufficient leafy spurge for the research blocks, and willing participation of the landowner(s).

4.2.2 Experimental Design

Additional information regarding the experimental design and setup are available in **Chapter 3: 3.2.2 Experimental Design.**

Annual changes in leafy spurge biomass and forage production were measured each year. Biomass was measured by clipping a subsample of the treatment plot (0.20 m x 1 m), bagging each plant functional group (leafy spurge, forbs, grasses, shrubs, and litter) separately, drying the material, and weighing each separately. In 2022 biomass and soil samples were collected from July 19 to August 10, and in 2023 biomass and soil samples were collected from July 6 to 19.

4.2.3 Sample Processing

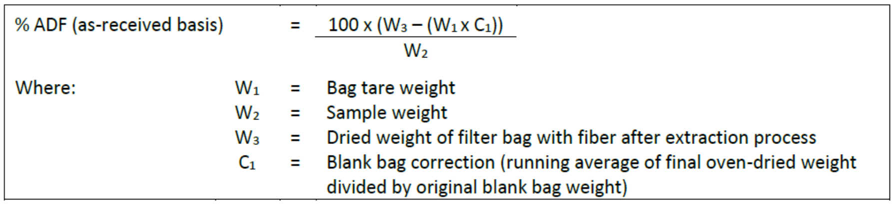
Leafy spurge and grass biomass were dried for a minimum of 72 hours at 60 ^{o}C, then weighed. Grinding of dried grass biomass was completed using a Model 4 Thomas Wiley single speed laboratory mill (manufactured by Troemner, Thorofare, NJ) with a 1 mm mesh screen size. Small grass biomass samples were ground using a Thomas Scientific Wiley single speed mini cutting mill (manufactured by Troemner, Thorofare, NJ) and a 1 mm mesh screen size. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis were completed on all grass biomass samples using the ANKOM^{DELTA} Automated Fiber Analyzer (ANKOM Technology, Macedon, NY). Grass biomass samples were also analyzed for nitrogen using a LECO® CN628 Elemental Determinator (LECO Corporation, St. Joseph, MI).

4.2.3.1 ANKOM^{DELTA} Automated Fiber Analyzer Methodology

The ANKOM^{DELTA} Automated Fiber Analyzer (ANKOM Technology, Macedon, NY) was used to determine the NDF and ADF of the ground grass biomass samples. F57 ANKOM Technology Filter Bags were labelled with each sample ID and weighed before and after adding approximately 0.5 g of the ground grass sample inside followed by heat sealing the bag. The weight (W_1) of the pre-filled bag, and the weight (W_2) with the added grass sample were recorded.

During processing, the filter bag allows for flow though of chemical solutions while retaining the non-soluble materials (Ankom Technology, 2020). Thus, the grass components are dissolved as they are subjected to the appropriate chemical (acid detergent or neutral detergent) solutions, leaving behind the desired fiber component (Ankom Technology, 2020). Samples are processed for 80 minutes for NDF and 70 mins for ADF (Vogel et al., 1999). Following completion of the sample processing in the ANKOM$^{\text{DELTA}}$ Automated Fiber Analyzer, filter bags were removed and excess water pressed from the bags. Bags were then soaked in a beaker filled with acetone for 3 to 5 minutes, then extracted from the acetone and gently pressed before placing them on a tray to air dry for 5 to 10 minutes (Vogel et al., 1999). The tray with the filter bags was then placed in the drying oven at 102 °C for a minimum of 3 hours followed by re-weighing (W_3) of the filter bags. Results were then determined gravimetrically using the equation shown in **(4.1)** for both ADF and NDF (Ankom Technology, 2020). NDF analysis was completed first for all filter bags followed by ADF analysis.

(4.1)



$$\text{\% ADF (as-received basis)} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$$

Where:
W_1 = Bag tare weight
W_2 = Sample weight
W_3 = Dried weight of filter bag with fiber after extraction process
C_1 = Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

4.2.3.2 LECO® CN628 Elemental Determinator Methodology

A LECO® CN628 Elemental Determinator (LECO Corporation, St. Joseph, MI) was used to measure the nitrogen content of each ground grass sample (LECO Corporation, 2022). A small amount, 0.1 g of each ground grass sample was placed into a 502-186 Tin Foil Cup and sealed. Sample mass was recorded and then placed in the appropriate position of the sample carousel. A leak check and calibration of the machine was performed each day before analyzing samples. Nitrogen results were then multiplied by 6.25 to obtain crude protein values.

4.2.4 Statistical Analysis

We included the structural variables from the experiment in all analyses unless otherwise noted; herbicide and fertilizer treatments and year were included as fixed factorial effects, with block and plot as random effects due to repeated measures. Due to large differences in soil texture among sites, we also used soil texture as a fourth factorial fixed effect variable. For this, we grouped the St. Denis and Grenfell together as loamy sites and the North Battleford and Manitou sites together as sandy sites.

Leafy spurge biomass, grass biomass, ADF forage quality, NDF forage quality, and forage protein were assessed using a mixed effects LMER model with the lme4 package (Bates et al., 2015), with the lmerTest package (Kuznetsova et al., 2017) used to estimate degrees of freedom and p-values for F-tests. Figures were generated using the afex package (Singmann et al., 2024). Leafy spurge biomass, grass biomass, ADF data, and NDF data were square root transformed to improve the normality of the residuals, while forage crude protein was log transformed. Additionally, plot was removed from the forage protein analysis because the model was too complex and the results of the ranova indicated that plot was not a significant random-effect term in the model. All statistical analyses were conducted in R version 4.3.3 (R Core Team, 2024).

4.3 Results

4.3.1 Effects of Herbicide and Fertilizer Treatments on Leafy Spurge Biomass

Herbicides, but not fertilizers, had significant effects on leafy spurge biomass. Herbicide effects, however, were dependent on both the soil texture and the year of the study (**Table 4.1**). 2,4-D provided strong control of leafy spurge in the first year; however, leafy spurge biomass recovered in the second year (**Figure 4.1**). Overdrive modestly reduced leafy spurge biomass in year one, but not year two. Navius and Tordon provided multi-year control as both products provide residual effects; however, Navius control was limited in year one due to an application error. There was also a herbicide by soil texture interaction, where leafy spurge biomass was lower in sandy soil in both years, except where the herbicide treatment reduced biomass to near zero in the loamy sites (**Figure 4.1**).

Table 4.1: Results of the mixed effects model on leafy spurge biomass with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	Sum Sq	Mean Sq	Df (num/den)	F value	P	Significance
Herbicide	34941	8735.20	4/280	195.876	< 0.001	***
Fertilizer	213	42.50	5/281	0.953	0.447	
Year	3418	3418.00	1/290	76.644	< 0.001	***
Soil Texture	689	689.10	1/10	15.452	0.003	**
Herbicide x Fertilizer	566	28.30	20/280	0.635	0.885	
Herbicide x Year	16439	4109.80	4/290	92.156	< 0.001	***
Fertilizer x Year	170	34.00	5/290	0.763	0.577	
Herbicide x Soil Texture	2277	569.20	4/280	12.763	<0.001	***
Fertilizer x Soil Texture	194	38.80	5/281	0.871	0.501	
Year x Soil Texture	382	382.40	1/290	8.574	0.004	**
Herbicide x Fertilizer x Year	561	28.10	20/290	0.629	0.890	
Herbicide x Fertilizer x Soil Texture	409	20.40	20/280	0.458	0.979	
Herbicide x Year x Soil Texture	1249	312.20	4/290	7.000	<0.001	***
Fertilizer x Year x Soil Texture	93	18.50	5/290	0.415	0.838	
Herbicide x Fertilizer x Year x Soil Texture	1265	63.20	20/290	1.418	0.112	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

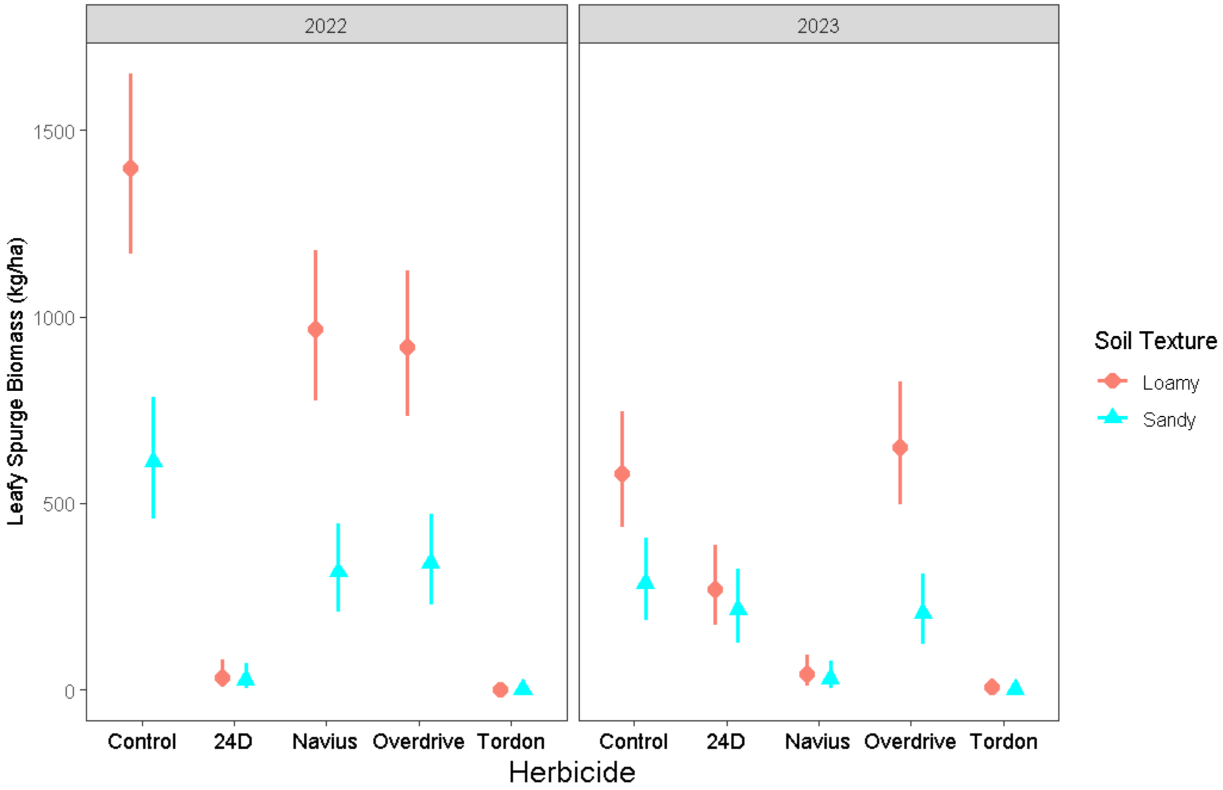


Figure 4.1: Effects of herbicide treatments on leafy spurge biomass (kg/ha) by soil texture and year. Each panel is divided by year. The symbols represent the estimated marginal mean leafy spurge biomass, while the bars represent the 95% confidence interval.

4.3.2 Effects of Herbicide and Fertilizer Treatments on Grass Biomass

Herbicides and fertilizers had significant effects on grass biomass. The effects of the fertilizers depended on soil texture, while herbicides were dependent on the interaction with either soil texture or year (**Table 4.2**). Grass biomass was increased with the use of herbicides, except Overdrive, with Navius and Tordon showing the greatest increase in both years compared to the unsprayed (**Figure 4.2**), especially in loamy soils (**Figure 4.3**). All fertilizer treatments increased grass biomass in loamy soils, though only N+P+Micro and NP treatments were significant. In sandy soils, the N treatments, including N, NP, and N+P+Micro, significantly increased grass biomass compared to the Control (**Figure 4.4**).

Table 4.2: Results of the mixed effects model on grass biomass with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	Sum Sq	Mean Sq	Df (num/den)	F value	P	Significance
Herbicide	5098.3	1274.6	4/277	29.441	<0.001	***
Fertilizer	2445.1	489.0	5/278	11.296	<0.001	***
Year	9007.2	9007.2	1/287	208.056	<0.001	***
Soil Texture	1163.2	1163.2	1/10	26.868	<0.001	***
Herbicide x Fertilizer	467.2	23.4	20/277	0.540	0.948	
Herbicide x Year	776.6	194.2	4/287	4.485	0.002	**
Fertilizer x Year	461.4	92.3	5/287	2.132	0.062	
Herbicide x Soil Texture	480.8	120.2	4/277	2.777	0.027	*
Fertilizer x Soil Texture	707.4	141.5	5/278	3.268	0.007	**
Year x Soil Texture	1376.8	1376.8	1/287	31.803	<0.001	***
Herbicide x Fertilizer x Year	516.2	25.8	20/287	0.596	0.914	
Herbicide x Fertilizer x Soil Texture	633.9	31.7	20/277	0.732	0.792	
Herbicide x Year x Soil Texture	315.9	79.0	4/287	1.824	0.124	
Fertilizer x Year x Soil Texture	50.4	10.1	5/287	0.233	0.948	
Herbicide x Fertilizer x Year x Soil Texture	568.9	28.4	20/287	0.657	0.866	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

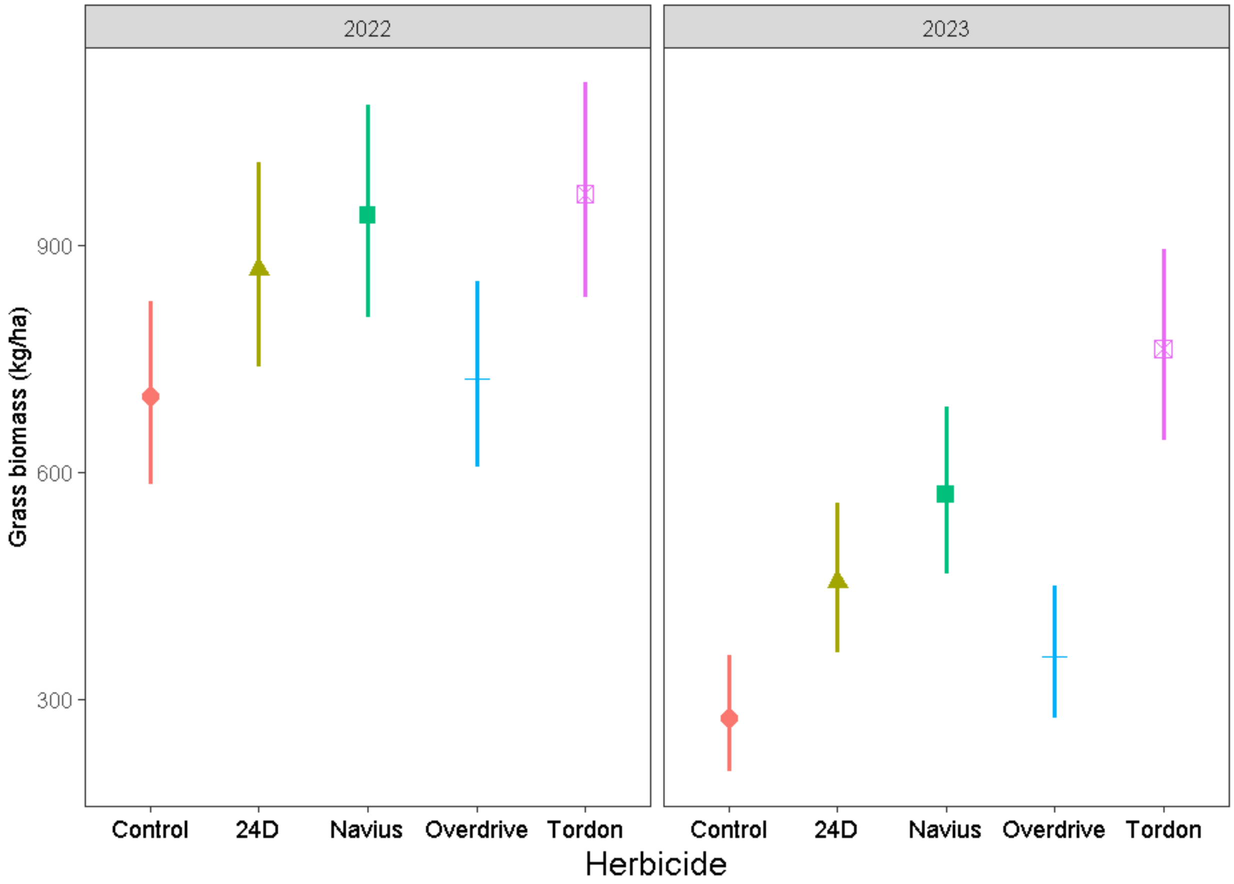


Figure 4.2: Effects of herbicide treatments on grass biomass by year. Each panel is divided by year. The symbols represent the estimated marginal mean grass biomass, while the bars represent the 95% confidence interval.

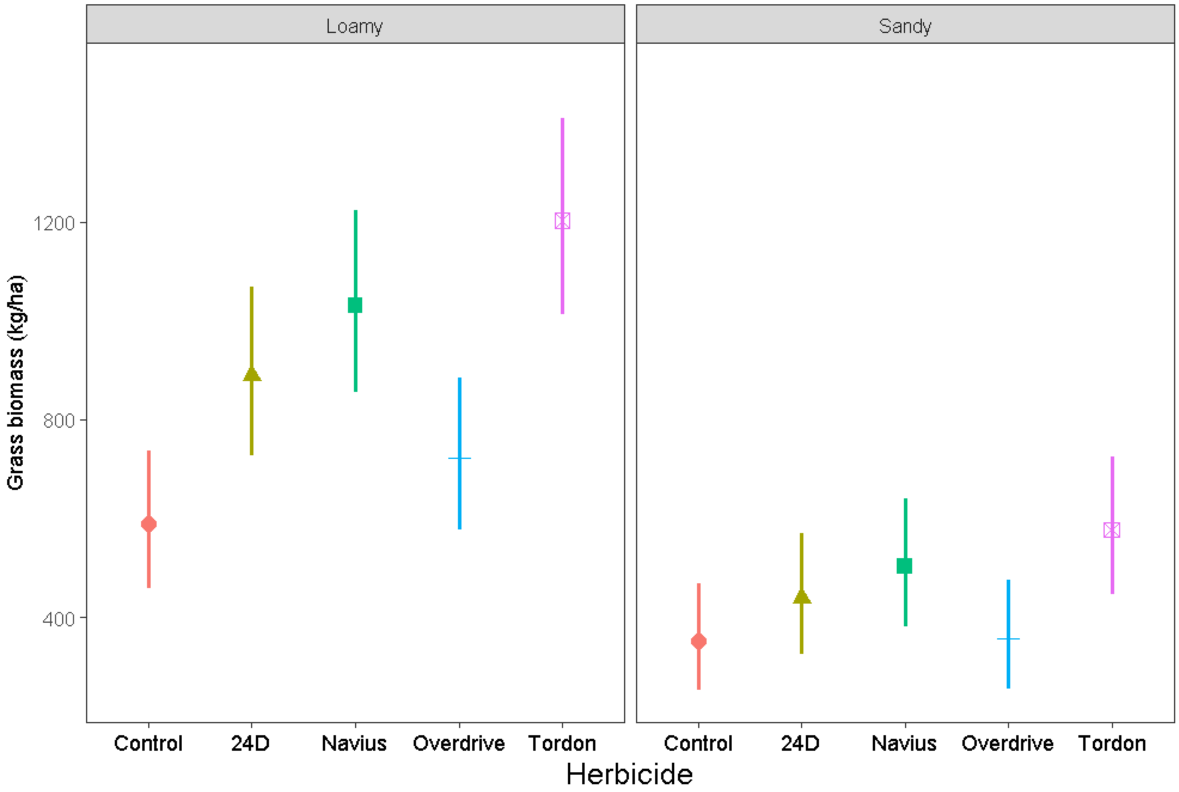


Figure 4.3: Effects of herbicide treatments on grass biomass by soil texture. Each panel is divided by soil texture. The symbols represent the estimated marginal mean grass biomass, while the bars represent the 95% confidence interval.

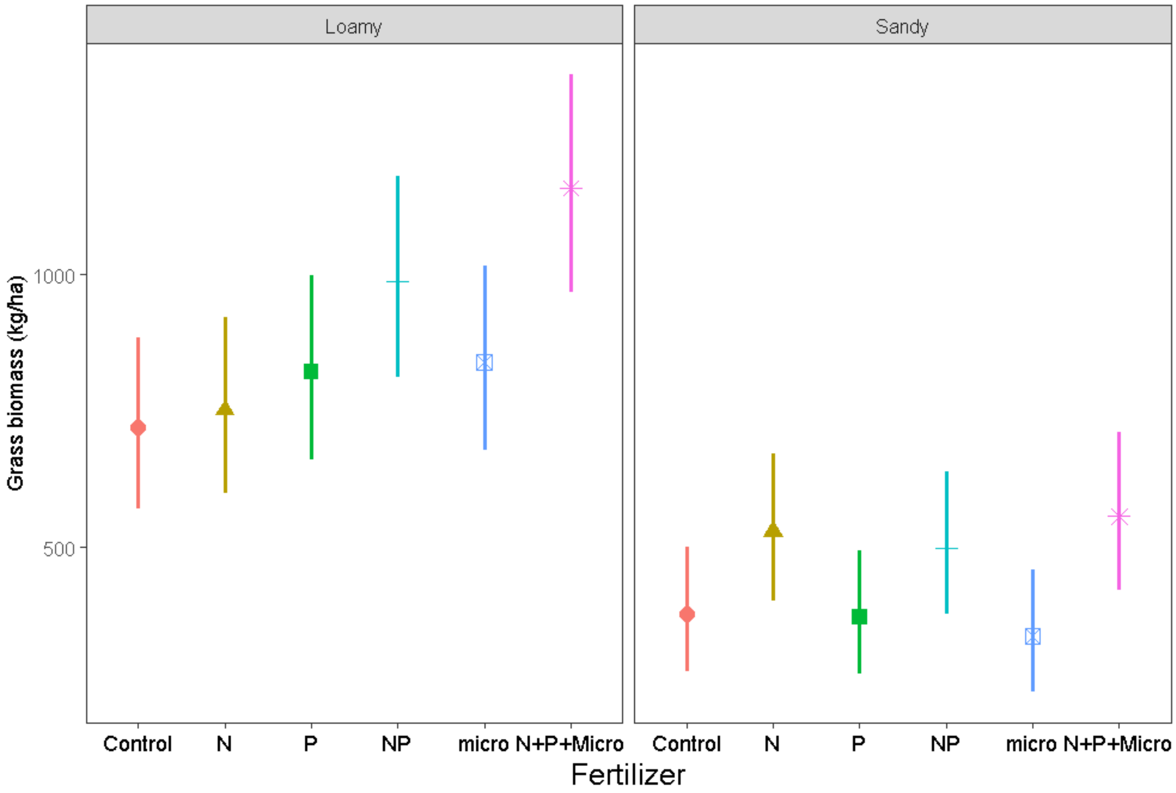


Figure 4.4: Effects of fertilizer treatments on grass biomass by soil texture. Each panel is divided by soil texture. The symbols represent the estimated marginal mean grass biomass, while the bars represent the 95% confidence interval.

4.3.3 Effects of Herbicide and Fertilizer Treatments on ADF

Herbicide, fertilizer, and year had significant effects on ADF (% dry matter) (**Table 4.3**) as main effects, with a marginal (P = 0.08) interaction effect with herbicide and fertilizer. Tordon showed reduced ADF (% dry matter) values (**Figure 4.5**) as did all N fertilizer treatments (including N, N+P+Micro, and NP) (**Figure 4.6**). The 2023 ADF (% dry matter) value was significantly lower compared to the 2022 value (data not shown). All N treatments (N, NP, and N+P+Micro) reduced ADF (% dry matter) with 2,4-D. The N and N+P +Micro treatments reduced ADF (% dry matter) with both Navius and Overdrive, while NP reduced ADF (% dry matter) with Tordon (**Figure 4.7**). Thus, N and N+P+Micro reduced ADF (% dry matter) in combination with 3 herbicide treatments 2,4-D, Navius and Overdrive, while NP reduced ADF (% dry matter) with Tordon.

Table 4.3: Results of the mixed effects model on ADF (% dry matter) forage quality with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	Sum Sq	Mean Sq	Df (num/den)	F value	P	Significance
Herbicide	0.566	0.141	4/278	3.760	0.005	**
Fertilizer	1.180	0.236	5/279	6.272	<0.001	***
Year	1.581	1.581	1/287	42.023	<0.001	***
Soil Texture	0.015	0.015	1/10	0.410	0.536	
Herbicide x Fertilizer	1.129	0.056	20/277	1.500	0.080	marginal
Herbicide x Year	0.214	0.054	4/287	1.426	0.225	
Fertilizer x Year	0.258	0.051	5/287	1.371	0.235	
Herbicide x Soil Texture	0.124	0.031	4/278	0.823	0.511	
Fertilizer x Soil Texture	0.105	0.021	5/279	0.557	0.733	
Year x Soil Texture	5.000e-04	5.000e-04	1/287	0.013	0.908	
Herbicide x Fertilizer x Year	0.518	0.026	20/286	0.689	0.837	
Herbicide x Fertilizer x Soil Texture	0.672	0.033	20/277	0.893	0.596	
Herbicide x Year x Soil Texture	0.160	0.040	4/287	1.067	0.373	
Fertilizer x Year x Soil Texture	0.129	0.026	5/287	0.684	0.636	
Herbicide x Fertilizer x Year x Soil Texture	0.600	0.030	20/286	0.797	0.717	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

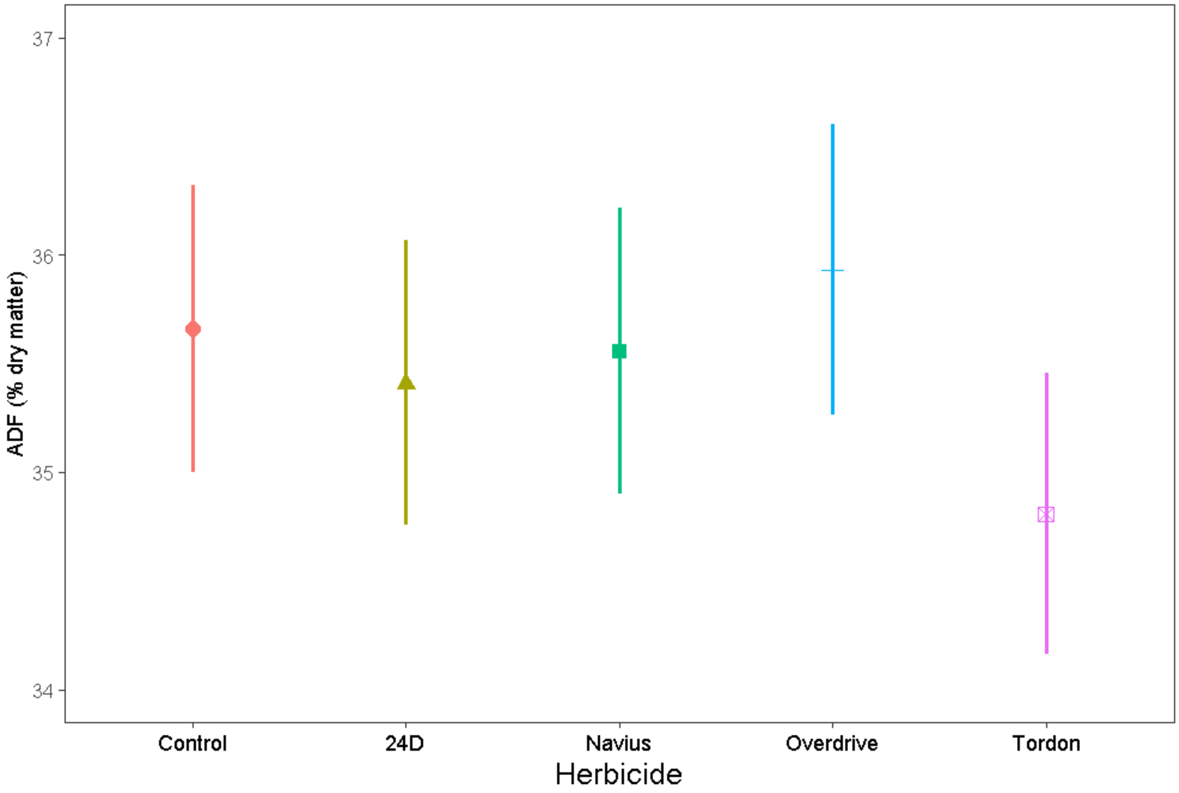


Figure 4.5: Effects of the herbicide treatments on ADF (% dry matter). The symbols represent the estimated marginal mean ADF (% dry matter), while the bars represent the 95% confidence interval.

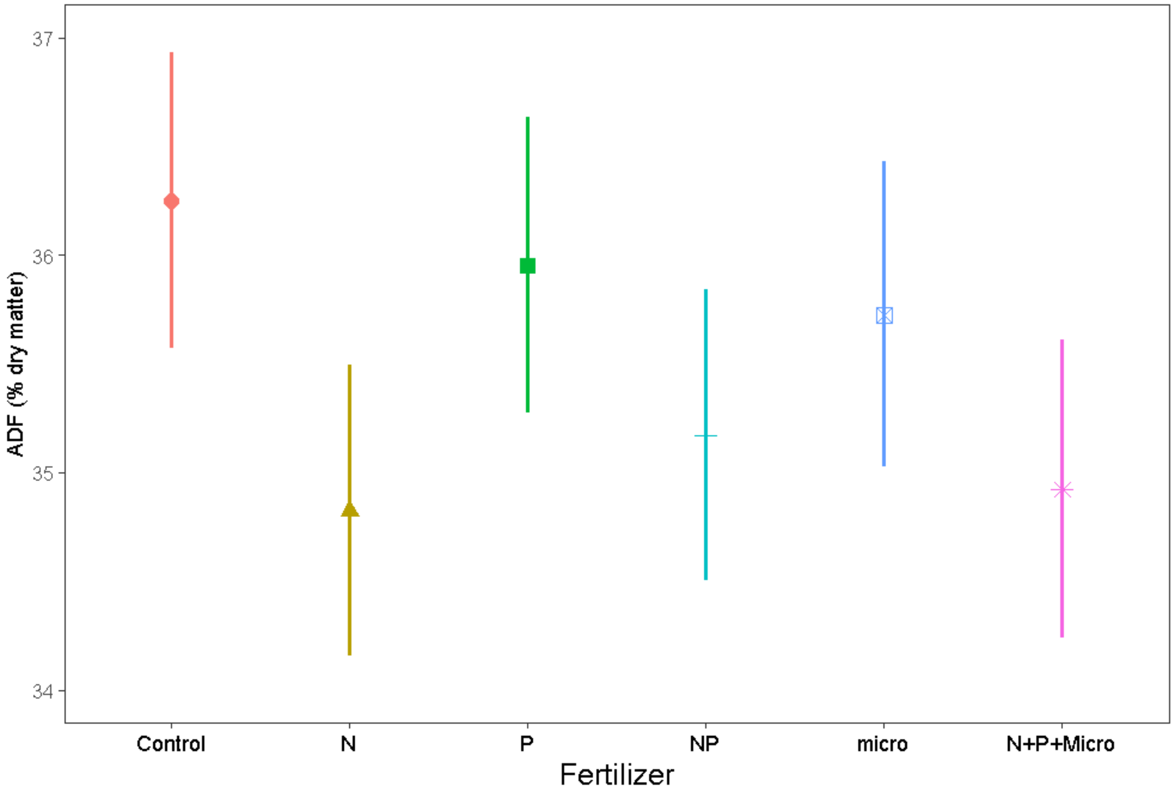


Figure 4.6: Effects of fertilizer treatments on ADF (% dry matter). The symbols represent the estimated marginal mean ADF (% dry matter), while the bars represent the 95% confidence interval.

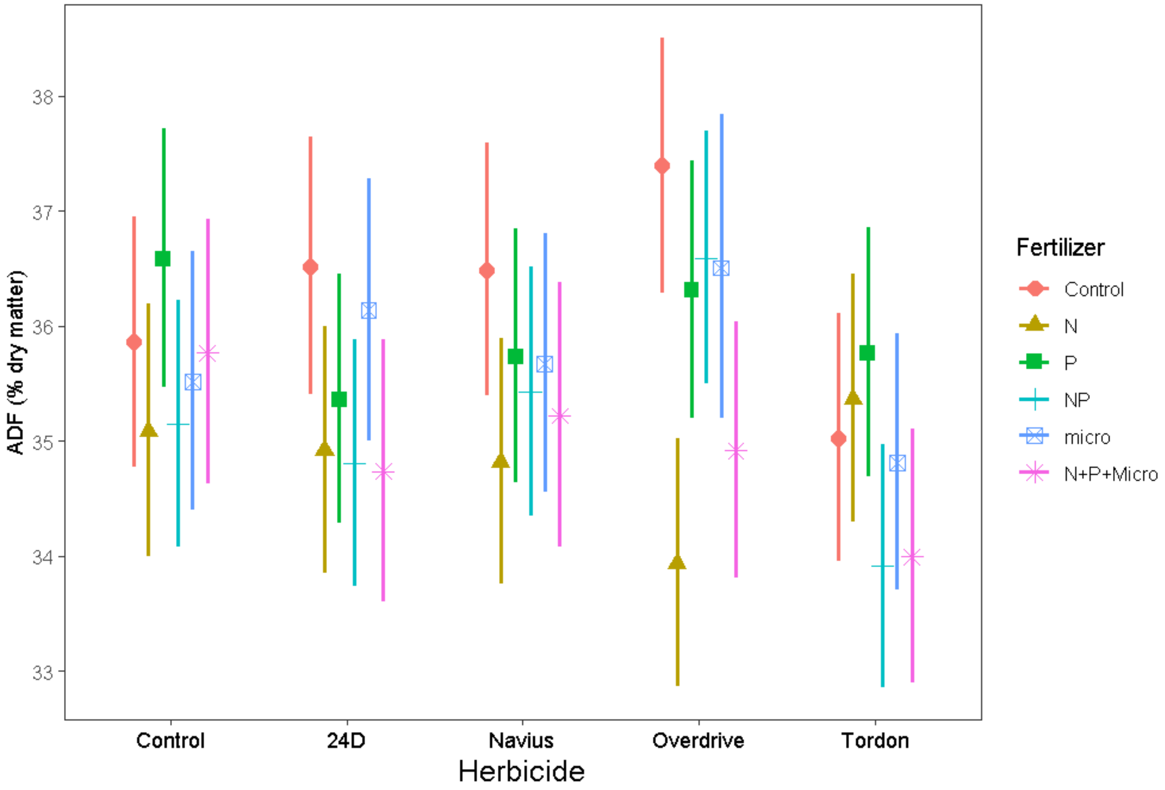


Figure 4.7: Marginal effects (P = 0.08) of herbicide and fertilizer treatments on ADF (% dry matter). The symbols represent the estimated marginal mean ADF (% dry matter), while the bars represent the 95% confidence interval.

4.3.4 Effects of Herbicide and Fertilizer Treatments on NDF

The interactive effect of herbicide and fertilizer was significant for NDF (% dry matter), as was the interactive effect of herbicide and year (**Table 4.4**). Phosphorus significantly increased NDF (% dry matter) in the Control, while NP and P decreased NDF (% dry matter) with 2,4-D; N+P+Micro decreased NDF (% dry matter) with Navius, and all fertilizer treatments, except micro, reduced NDF (% dry matter) with Overdrive (**Figure 4.8**). All herbicide treatments except Navius had lower NDF (% dry matter) in year two (**Figure 4.9**).

Table 4.4: Results of the mixed effects model on NDF (% dry matter) with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	**Sum Sq**	**Mean Sq**	**Df (num/den)**	**F value**	**P**	**Significance**
Herbicide	0.363	0.091	4/282	3.364	0.010	*
Fertilizer	0.448	0.090	5/283	3.324	0.006	**
Year	3.460	3.460	1/290	128.317	<0.001	***
Soil Texture	0.519	0.519	1/10	19.267	0.001	**
Herbicide x Fertilizer	0.999	0.050	20/281	1.853	0.016	*
Herbicide x Year	0.470	0.117	4/289	4.358	0.002	**
Fertilizer x Year	0.049	0.010	5/289	0.362	0.874	
Herbicide x Soil Texture	0.185	0.05	4/282	1.717	0.146	
Fertilizer x Soil Texture	0.134	0.03	5/283	0.995	0.421	
Year x Soil Texture	0.782	0.782	1/290	29.003	<0.001	***
Herbicide x Fertilizer x Year	0.284	0.014	20/288	0.527	0.954	
Herbicide x Fertilizer x Soil Texture	0.576	0.029	20/281	1.069	0.382	
Herbicide x Year x Soil Texture	0.131	0.033	4/289	1.211	0.306	
Fertilizer x Year x Soil Texture	0.079	0.016	5/289	0.588	0.709	
Herbicide x Fertilizer x Year x Soil Texture	0.617	0.031	20/288	1.144	0.303	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

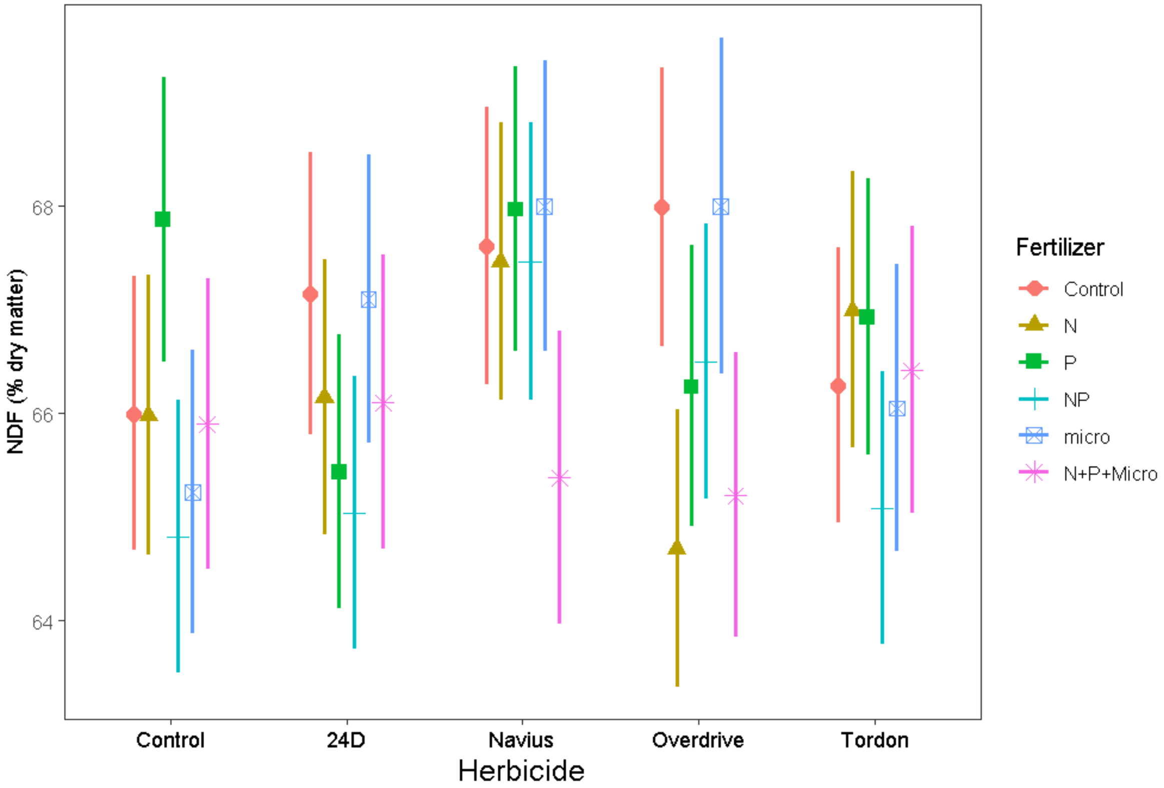


Figure 4.8: Effects of the herbicide and fertilizer treatments on NDF (% dry matter). The symbols represent the estimated marginal mean NDF (% dry matter), while the bars represent the 95% confidence interval.

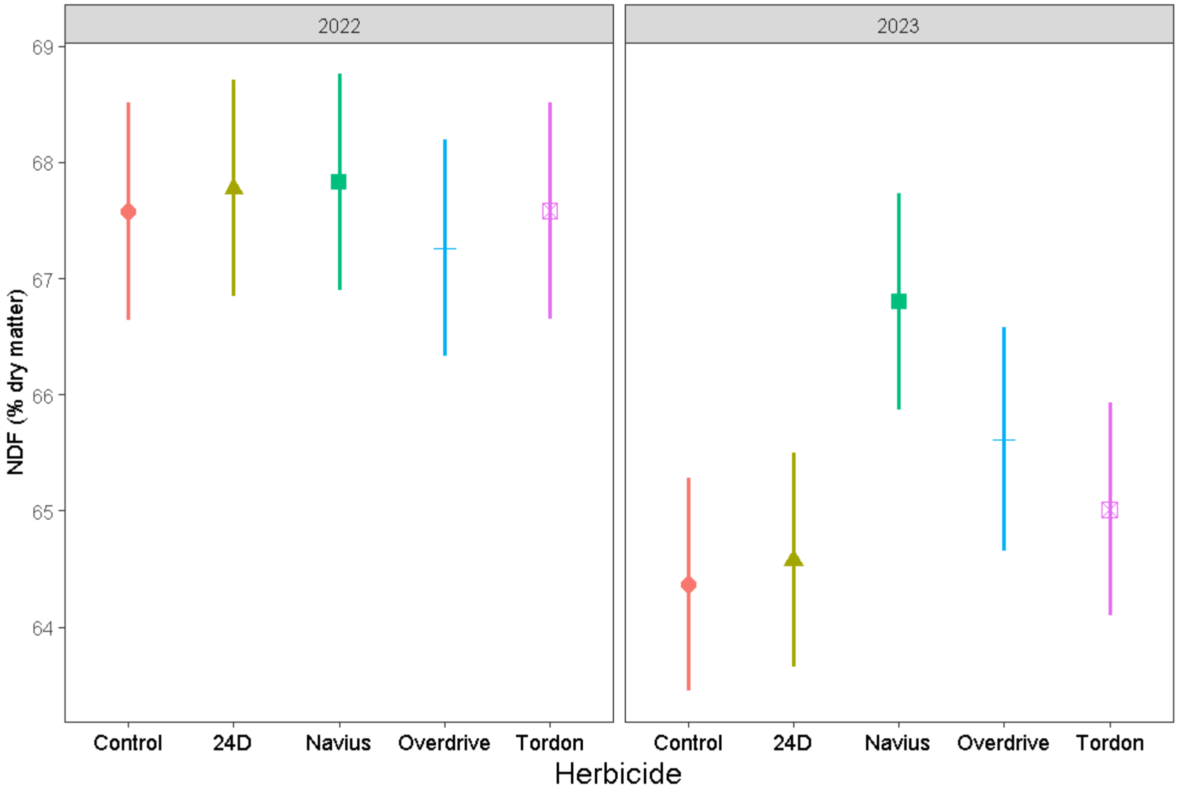


Figure 4.9: Effects of the herbicide treatments on NDF (% dry matter) by year. Each panel is divided by year. The symbols represent the estimated marginal mean NDF (% dry matter), while the bars represent the 95% confidence interval.

4.3.5 Effects of Herbicide and Fertilizer Treatments on Forage Crude Protein

Fertilizer had significant effects on forage crude protein values (dry matter conversion), while herbicide effects depended on the year and soil texture (**Table 4.5**). As expected, the N treatments (N, NP, and N+P+Micro) all increased the forage crude protein level (dry matter conversion) in both years, with 2023 showing a greater increase in values (**Figure 4.10**). All herbicides, except Overdrive, increased crude protein % (dry matter conversion) in both soil types in both years, however the effect of 2,4-D was no longer detectable in sandy soil in year two (**Figure 4.11**).

Table 4.5: Results of the mixed effects model on LECO crude protein values with herbicide, fertilizer, year, and soil texture as explanatory variables.

Variable	**Sum Sq**	**Mean Sq**	**Df (num/den)**	**F value**	**P**	**Significance**
Herbicide	2.258	0.564	4/561	22.470	<0.001	***
Fertilizer	13.408	2.682	5/562	106.760	<0.001	***
Year	0.002	0.002	1/561	0.083	0.773	
Soil Texture	0.002	0.002	1/10	0.089	0.771	
Herbicide x Fertilizer	0.522	0.026	20/561	1.039	0.413	
Herbicide x Year	0.402	0.101	4/561	4.006	0.003	**
Fertilizer x Year	1.891	0.378	5/561	15.057	<0.001	***
Herbicide x Soil Texture	0.330	0.082	4/561	3.284	0.011	*
Fertilizer x Soil Texture	0.147	0.029	5/562	1.170	0.322	
Year x Soil Texture	0.384	0.384	1/561	15.293	<0.001	***
Herbicide x Fertilizer x Year	0.315	0.016	20/561	0.628	0.893	
Herbicide x Fertilizer x Soil Texture	0.406	0.020	20/561	0.809	0.704	
Herbicide x Year x Soil Texture	0.260	0.065	4/561	2.585	0.036	*
Fertilizer x Year x Soil Texture	0.054	0.011	5/561	0.430	0.828	
Herbicide x Fertilizer x Year x Soil Texture	0.184	0.009	20/561	0.367	0.995	

*Significance '***' 0.001 '**' 0.01 '*' 0.05.*

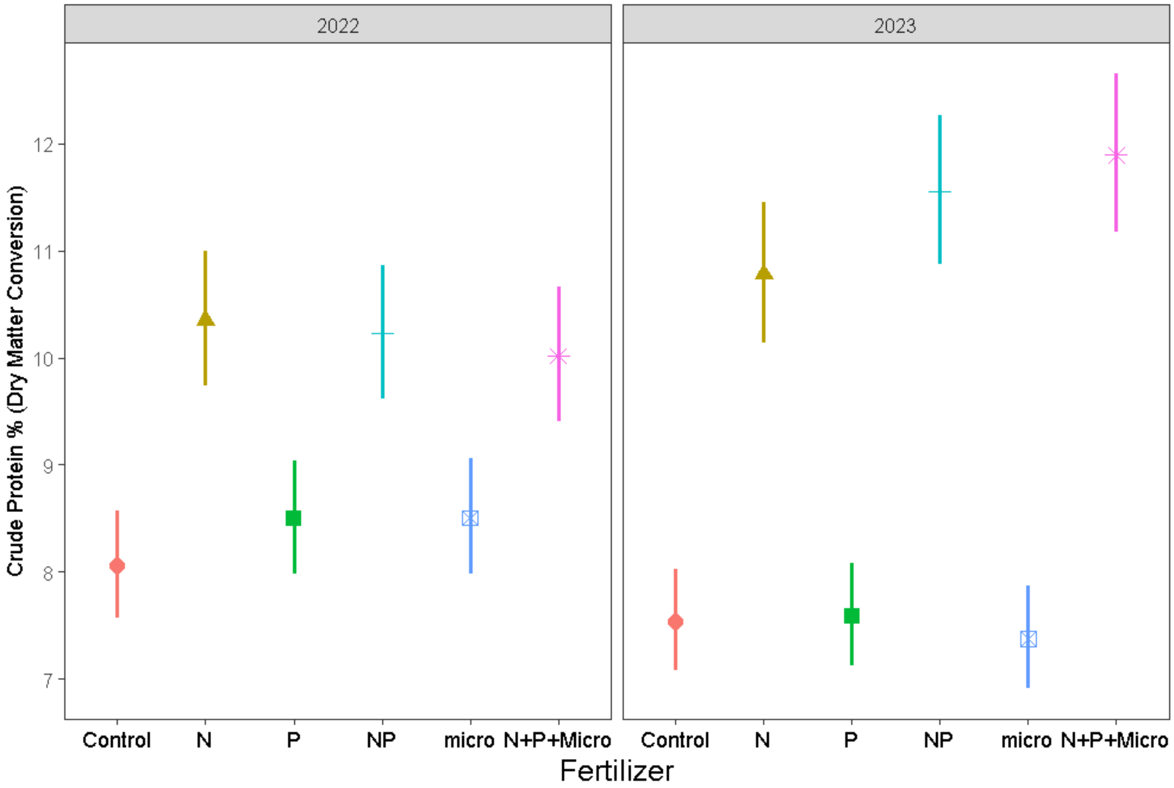


Figure 4.10: Effects of fertilizer treatments on crude protein % (dry matter conversion) by year. Each panel is divided by year. The symbols represent the estimated marginal mean crude protein % (dry matter conversion), while the bars represent the 95% confidence interval.

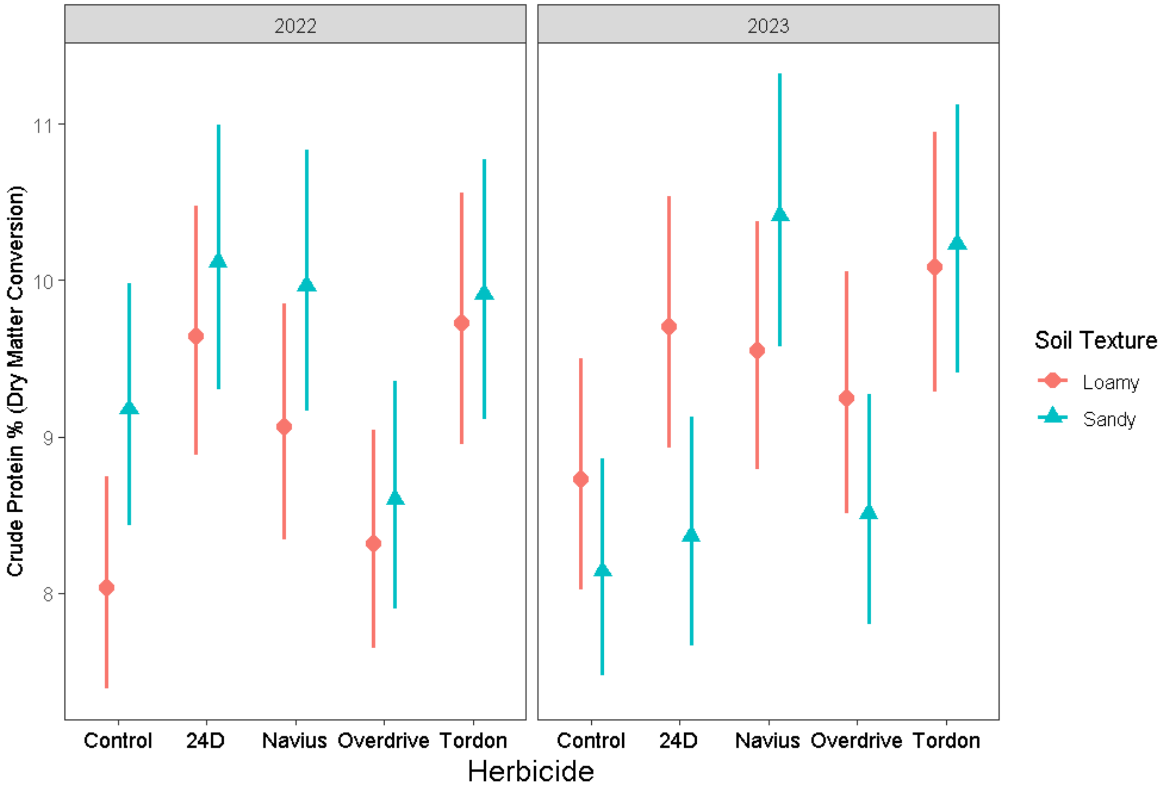


Figure 4.11: Effects of herbicide treatments on crude protein % (dry matter conversion) by year and soil texture. Each panel is divided by year. The symbols represent the estimated marginal mean crude protein % (dry matter conversion), while the bars represent the 95% confidence interval.

4.4 Discussion

All herbicide treatments decreased 2022 leafy spurge biomass compared to the control, with Navius and Overdrive treatments less effective due to the initial application error. Although a second treatment using the correct surfactant amount was applied, the leafy spurge was at an advanced stage of growth at the time. Both Navius (Bayer CropScience Inc., 2020) and Overdrive (BASF Canada Inc., n.d.-b) recommend application be completed during an active growth stage, thus the second treatment was rather late as the leafy spurge plants had largely completed their growth for the season. Additionally, Overdrive only offers top growth suppression of leafy spurge (BASF Canada Inc., n.d.-b), suggesting the herbicide is generally less effective on this plant species. In the second year, efficacy declined for 2,4-D and Overdrive as these herbicides offer limited residual effects. By contrast, Navius and Tordon offered multi-year control because of the residual effects of these herbicides, which extend weed control due to their persistence in the soil (Helling, 2005).

Herbicide treatments increased grass biomass in both years, more in 2022 than 2023, and more in loamy soils than sandy soils, with Tordon and Navius showing the greatest increases. Our findings are supported by multiple other studies that also found herbicide treated plots showed a marked increase in grass biomass (Bork et al., 2007; Cinar et al., 2013; Greet et al., 2016; Ortega & Pearson, 2010; Skurski et al., 2013). Our observed increase in grass biomass following herbicide application, especially with Navius and Tordon, highlights the benefit of controlling the competitive influence of leafy spurge. Grasses are not impacted by the selective herbicides and can quickly colonize and establish in the space formerly occupied by the invasive forb. As the canopy opened following the selective herbicide treatments at our sites, the reduced cover of leafy spurge gave space for grasses to increase and expand resulting in increased grass biomass.

The observed differences in herbicide activity in differing soil textures for leafy spurge and grass biomass can be explained through the complex interactions between the chemicals and the soil environment (Helling, 2005). As discussed in Chapter 3, soil texture is strongly linked to variations in soil properties including soil moisture and fertility levels, which in turn can alter herbicide efficacy. Leafy spurge biomass was lower in sandy soils compared to loamy soils across all herbicide treatments, while grass biomass was higher in loamy soils compared to sandy soils across all herbicide treatments, especially with Navius and Tordon. Compared to sandy soils, loamy soils increase water retention (Pauwels et al., 2023) and in a water limited environment, like the Northern Great Plains, increased moisture in loamy sites may have allowed increased grass growth relative to sandier sites once competition was relaxed by leafy spurge suppression (Sanaei et al., 2019). Further, a recent study by Pauwels et al. (2023) found AMF can act as a soil conditioner. They observed a decrease in water retention in loamy soils with AMF, while in sandy soils water retention was increased. Thus, complex differences occur within different soil textures resulting in varying herbicide effect outcomes.

The reduced response of grass biomass to herbicide treatments in 2023 than in 2022 can be explained through annual environmental variation. Interannual variation in productivity is most often associated with weather, including fluctuations in rainfall amounts and temperatures

(Fuhlendorf et al., 2009; Werner et al., 2020). Additionally, herbicide and fertilizer use have been found to yield significantly different outcomes depending on the conditions when applied (Werner et al., 2020). Multi-year drought conditions were experienced throughout much of Saskatchewan over the study period (Saskatchewan Water Security Agency, 2022; Saskatchewan Water Security Agency, 2023), including at our experimental sites. Fuhlendorf et al. (2009) found that up to 90% of the annual variation in canopy cover of grasses was due to a year effect rather than the effects of the herbicide application. Further, prolonged droughts extending over multiple years can cause depletion of soil moisture and greatly reduce productivity (Meisser et al., 2019). Consequently, the efficacy of herbicide as a means of increasing forage production will depend on the year in which it is applied and the recent precipitation history.

In general, fertilizer treatments are expected to increase forage yields (Bedaso et al., 2022). Nitrogen is known to improve forage yield and enhance the competitive ability of grasses against invasive forbs (Bork et al., 2007); however, soil texture can influence soil fertility and nutrient management, with N more easily leached from sandy soils (Manjula, 2017). In our study, N fertilizer treatments, especially NP and N+P+Micro treatments, increased grass biomass, particularly in loamy soils, which we assume is less water limited. The increase in grass growth in treatments containing both N and P also suggest that loamy grasslands may be co-limited by these two nutrients, as is common in many grasslands worldwide (Fay et al., 2015).

Herbicide and fertilizer effects on forage quality were complex, with treatment specific results. Both ADF and NDF were influenced by an interactive effect with herbicide and fertilizer, with a significant influence on NDF and a marginal effect on ADF. The reasoning for these complex interactions is unclear but could be due to the specific chemistries of the products. Nevertheless, these varying results suggest that the application of herbicides and fertilizers and impacts to forage quality are influenced by site specific factors such as soil and weather conditions in addition to the vegetation type and growth stage of the forage being assessed (Faji et al., 2022).

In our study, only Tordon effectively reduced ADF (% dry matter) on its own, while other studies found that treatments containing metsulfuron reduced ADF (% dry matter) (Israel et al., 2016; Sather, Roberts, et al., 2013). We found no effect with Navius, our only herbicide with metsulfuron as an active ingredient. However, Sather, Roberts, et al. (2013) determined that ADF only decreased with metsulfuron-containing herbicides when applied at the boot stage. Given our initial Navius application was ineffective and reapplied far after the boot stage, it is possible that reductions in ADF may have occurred if the treatment was applied as intended. Although other herbicides had no significant impact on ADF (Bork et al., 2007; Cinar et al., 2013), it is possible that proper application of Tordon just prior to flowering for many native grasses acted similarly to boot stage application of metsulfuron (Sather, Roberts, et al., 2013). It may also be that the strong initial effects of Tordon on leafy spurge allowed plants to grow more quickly and thus they invested less in structural tissues represented by ADF.

Similar to others (Cinar et al., 2013; Daşci & Çomakli, 2011; Delevatti et al., 2019), we found that fertilizer, especially N fertilizer treatments (including N, NP, and N+P+Micro), decreased ADF values resulting in increased forage quality. This decrease in ADF has been ascribed to the increase in accumulated crude protein and other soluble contents in the cell wall, resulting in dilution of the cell wall (Delevatti et al., 2019). Essentially, in order for the forage plant to increase crude protein content, it must allocate its resources to protein synthesis rather than producing cell wall structural components resulting in reduced ADF values (Delevatti et al., 2019). However, Bork et al. (2007) found fertilizer treatments generally resulted in lower forage quality as ADF levels were increased relative to their unfertilized control plots.

All herbicides, except Overdrive, increased protein values in both years and both soil types, however the effect of 2,4-D in sandy soils was no longer detectable in year two likely due to leafy spurge recovery and increased competition for available soil N. The increase in grass protein values may be a consequence of effective herbicide control of leafy spurge and other broadleaf species which produces added dead plant material and contributes to more decomposition and N added to the soil. Leafy spurge litter is also known to contain more N than other forb and grass species (McTee et al., 2017). Further, leafy spurge litter is known to decompose faster than native and invasive grasses and leafy spurge invaded areas are associated with higher soil N availability than native plants, possibly as a result of a faster turnover of litter (McTee et al., 2017). Together these may have contributed to the increase in grass protein values. In contrast to our findings, data from other Canadian pastures found that herbicides had no significant effect on grass protein values (Bork et al., 2007). Metsulfuron treated forage has been found to have lower stem yields compared to non-treated forage resulting in greater crude protein as stems have higher fibre and less crude protein than leaves (Israel et al., 2016). These results may be herbicide specific, however, as forage from metsulfuron containing herbicide treatments had greater crude protein values than the nontreated control and the picloram + 2,4-D treatment in other studies (Sather, Roberts, et al., 2013). This suggests that only the Navius herbicide should have increased protein content; however, it is unclear why all herbicides were similarly effective here. Previous work has attributed variability in forage protein to legume abundances and variability in N fixation (Cinar et al., 2013). Herbicides can vary in their effects on the legume component (Miller et al., 2015), which may explain previous variation in herbicide effects on forage protein. Our sites had few legumes, so their loss likely would not have affected grass protein. Alternatively, the use of fertilizer in many pastures may reduce nitrogen limitation and thus increases in N following herbicide may be negligible relative to background N levels in many studies (Cinar et al., 2013). We found no interactions between herbicide and fertilizer; however, despite all N fertilizer treatments increasing forage protein, suggesting this latter explanation is unlikely.

4.5 Conclusion

Forage quality and quantity are important considerations for livestock producers looking to use herbicides and fertilizers to control invasive plant species. Certainly, decreasing either of these valuable resources would not be an ideal outcome and thus it was important that we confirm the impact of treatments as part of this study. Our results demonstrated that herbicides decrease leafy spurge biomass while increasing grass biomass, especially in loamy soils. Removing the competition of the invasive plant species increases available space, light, and nutrients for the grasses to expand their presence. In terms of forage quality, Tordon and N fertilizer treatments both independently decreased ADF, while other fertilizer and herbicide combinations helped decrease NDF; NP fertilizer combined with 2,4-D; N+P+Micro fertilizer combined with Navius herbicide; and N or N+P+Micro fertilizers combined with Overdrive herbicide helped decrease NDF results versus use of the herbicide alone. As expected, N fertilizer treatments increased forage crude protein results, while Navius improved forage crude protein in sandy soils. Our results demonstrate that herbicides and fertilizers used independently and in combination can work together to control invasive plant species while improving forage quality and/or forage quantity, though the impacts to forage quality may be influenced by site specific factors.

Chapter 5: Synthesis and Conclusion

Invasion of grasslands by leafy spurge [*Euphorbia virgata* Wald & Kit (previously known as *Euphorbia esula* L.) see Flora of North America (Riina et al., 2020)] causes both ecological and economic concerns. Ecologically, leafy spurge contributes to the degradation of the grassland ecosystem by reducing native plant richness and diversity and modifying soil conditions by shifting soil microbial communities and altering nutrient availability (Gibbons et al., 2017; Liu et al., 2023). These alterations to the structure and function of the grassland ecosystem can result in economic losses through reduced forage yield, degraded forage quality, decreased carrying capacity, reduced livestock performance, and diminished land values (Cason & Sleugh, 2023; DiTomaso, 2000; Sather, Kallenbach, et al., 2013; Sleugh et al., 2023). Leafy spurge cover also influences altered forage utilization by cattle as most livestock avoid eating leafy spurge because of the milky latex it produces, which can irritate the skin and aggravate the digestive tracts of animals (Hein & Miller, 1992; St. John & Tilley, 2014). Consequently, livestock producers and land managers alike often desire to rid their lands of leafy spurge. Unfortunately, controlling leafy spurge is extremely challenging due to its vigorous clonal character and strong competitive abilities (Gucker, 2010; Messersmith et al., 1985).

Management actions to control leafy spurge often have negative side effects with some actions causing as much harm as the invasive plant to the resident plant community (Rinella et al., 2009). However, these negative control effects may be offset by positive effects from the native species as they rebound following management suppression efforts (Rinella et al., 2009). In effect, invasive plant management can have both positive and negative consequences for the native plant community making it challenging for land managers to determine whether control efforts are worthwhile (Rinella et al., 2009). Although abandoning efforts to control leafy spurge is not recommended, it is important to give consideration to the impacts of leafy spurge combined against the negatives and positives of control efforts (Skurski et al., 2013).

The use of herbicides for control of invasive species, including leafy spurge, often results in ecological trade-offs such as improved grass productivity as a result of reduced invasive weed pressure but at the cost of native forb species loss (Bork et al., 2007). This was true in our study where we observed decreased leafy spurge cover and biomass along with an increase in grass biomass and cover in herbicide treated plots (except Overdrive); however, native forb and shrub species cover loss occurred as a result. Although invasive grasses were rare at our sites, we also noted that herbicide use increased their abundance when they were present, suggesting the potential for further negative effects even though we could not statistically detect this effect in this study. Herbicide use improved forage quality as ADF decreased with the use of Tordon; although, Navius caused an increase in NDF and crude protein, suggesting that herbicide effects on forage quality are likely herbicide dependent. Thus, the application of broadleaf selective herbicides needs to be weighed against management objectives, such as the tolerance for loss of species richness with native forb and shrub species (Skurski et al., 2013). Additionally, sites invaded with invasive grass species, such as Kentucky bluegrass (*Poa pratensis*) and smooth bromegrass (*Bromus inermis*), may experience additional harm as herbicide treatment often facilitates increased invasion (Endress et al., 2012; Skurski et al., 2013). However, if the primary management goal is maximizing grass biomass for forage, then herbicide treatment may be effective and beneficial. Consideration must also be given to the impacts with forage quality as certain herbicides resulted in improved forage quality in some respects while decreasing forage quality in others.

Although fertilizers did not enhance herbicide effects on leafy spurge at smaller scales in the first two years of this study, at the larger plot level, N and micronutrient fertilizers showed increased control compared to the NP and N+P+Micro treatments, though only marginally compared to the Control. This difference in outcome with leafy spurge control between plot level and quadrat level fertilizer effects may be a scale dependent effect as a result of multiple ecological processes differentially impacting outcomes at different special scales (Tarasi & Peet, 2017). At larger spatial scales, greater environmental heterogeneity and microhabitat spaces provide favourable conditions for both invasive and native plants (Hill & Fischer, 2014), which allows for increased invasive species presence compared to smaller spatial scales (Tarasi & Peet, 2017). Effectively, invasive plants are more frequent in large scale plots than smaller scale plots. Thus, as scale continues to decrease, space becomes a limiting factor in individual abundance and spatial scales of measurement can result in dissimilar outcomes (Hill & Fischer, 2014).

Fertilizers also affected AMF colonization and soil nutrient composition, while increasing forage quantity and quality. Both forage quality and quantity were improved with N fertilization treatments. Grass biomass was increased with NP and N+P+Micro treatments, particularly in loamy soils, with all N treatments reducing ADF. As hypothesized, micronutrient and P fertilizers decreased initial AMF infectivity, but only when applied with 2,4-D and Tordon respectively, which also decreased the abundance of the host plant leafy spurge. Conversely, N fertilizer increased colonization when applied with residual herbicides, Navius and Tordon. This suggests that it may be possible to spuriously manage AMF colonization and ultimately enhance control of leafy spurge by combining herbicides and fertilizers, though further research is required.

The integration of herbicides and fertilizers is likely to be more beneficial in plant communities with a high percentage of native grasses, while caution may be needed in plant communities with a high presence of forbs or shrubs, or where there is an existing presence of invasive grasses (Cole et al., 2007). Fertilizer treatments may be less effective as an invasive plant management tool in years when insufficient moisture is received or at sites where the established plant community does not respond to the fertilizer treatment, such as forb dominated communities which do not perform as well as grass dominated ones (Cole et al., 2007). Caution should be exercised when applying fertilizer where invasive plant density is high as this may result in enhanced invasive plant growth over the native plant growth (Cole et al., 2007). Thus, it is important to consider the land management goals, the existing plant community, tolerance for loss of non-target species, and potential increase in invasive grasses when implementing leafy spurge control.

Results in response to the project objectives:

1. Navius and Tordon maximized control of leafy spurge control in both soil textures, however these residual herbicides were also associated with the greatest impacts to native forbs and shrubs. 2,4-D only provided first year control of leafy spurge due its lack of residual properties while providing the least impact to the native forbs and shrubs. At the plot level, micronutrient and N fertilizers reduced leafy spurge cover compared to other fertilizer treatments, though not compared to the Control, however, the direction does appear to be trending lower.
2. Navius and Tordon provided the greatest increase in grass productivity while effectively controlling leafy spurge, especially in loamy soils. NP and N+P+Micro fertilizer treatments increased grass biomass in loamy soils, while all N treatments (N, NP, and N+P+Micro) increased grass biomass in sandy soils.

Results in response to the hypotheses:

1. Contrary to the hypothesis that P would reduce leafy spurge abundance by reducing AMF colonization, we saw that P enhanced phytometer colonization in unsprayed plots. However, when combined with certain herbicide treatments, P was effective in enhancing leafy spurge control. All fertilizer treatments, except N+P+Micro, reduced phytometer colonization with 2,4-D, with the greatest decrease in phytometer colonization realized with 2,4-D and micronutrient and with Tordon and P. Thus, P and micronutrient fertilizers, when combined with certain herbicides, were effective in reducing AMF colonization. Conversely, the 2023 colonization data which analyzed colonization on collected leafy spurge plants found that all fertilizer treatments increased colonization compared to the Control.
2. Our results supported the hypothesis that the plant community would be altered as a result of the herbicide applications, including reduced plant diversity and cover of leafy spurge, native forbs, and native shrubs. Native forbs were significantly reduced by herbicide treatments, with Navius and Tordon showing the greatest decline. Shrubs were also reduced by herbicide use, though this was limited to sandy soils. It should be noted that this was a two-year study and thus provides effects over the short term and may not predict longer term outcomes.
3. As predicted, all fertilizer treatments increased grass biomass. In loamy soils only NP and N+P+Micro treatments were significant while all N treatments (N, NP, and N+P+Micro) were significant in sandy soils compared to the Control.

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