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MS ENVIRONMENTAL BIOLOGY CAPSTONE PROJECT

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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

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MS ENVIRONMENTAL BIOLOGY
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by

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CHAPTER 1. LITERATURE REVIEW

Mechanisms of Introduction and Management Techniques for Yellow Flag Iris (*Iris pseudacorus*)

Introduction

The effects and consequences of invasive species' introduction into native ecosystems are of increasing importance to ecologists and conservationists (Gallego-Tévar et al 2022, Hayasaka et al. 2018, Simberloff 2021). When a species is introduced in a novel ecosystem, there can be a multitude of consequences such as loss of species, decreases in biodiversity, and degradation of ecosystem services (Havel et al. 2015, Hershner & Havens 2008). Human-mediated plant introduction has been an area of particular concern because introduced plants can alter ecosystem functions and outcompete native vegetation (Lehan et al. 2013). When some species escape human cultivation, they can become invasive in natural ecosystems and degrade the natural habitat (Gallego-Tévar et al 2022, Hulme et al 2018).

One increasingly common invasive species is the yellow flag iris (*Iris pseudacorus*), an emergent aquatic perennial that has become a concern in mesic to aquatic ecosystems around the world (Global Invasive Species Database 2018, U.S Fish and Wildlife Service 2019, Xiong et al. 2023). The yellow flag iris displays large, showy yellow flowers on upright stems with long smooth leaves. To reproduce, the yellow flag iris propagates both sexually through seed pods and by rhizomes (Xiong et al. 2023). At the time of fruiting, seedpods can produce as many as 120 seeds each. (U.S Fish and Wildlife Service 2019, Morgan et al. 2018). Rhizomatous reproduction allows the iris to form large, dense stands that can proliferate throughout habitats and outcompete other vegetation. Whereas the production of seeds allows for the establishment of new stands.

The yellow flag iris often inhabits moist to wet areas, often in wetlands, along streams, and in brackish habitats (U.S Fish and Wildlife Service 2019). Yellow flag iris can also establish in disturbed places along dammed areas and urban ponds (Hayasaka et al. 2018, Thomson et al. 2021). Yellow flag iris is native to Europe, Asia and north Africa but has been cultivated for ornamental and wetland-restoration purposes worldwide, including North America, Japan, China, South America, South Africa, and Australia (Global Invasive Species Database 2018, Minuti et al. 2023, Xiong et al. 2023). Yellow flag iris was purposefully introduced as an ornamental species in the United States around 1850 (Lehan et al. 2013) and was first observed in the state of New York in 1868 however it is not defined weather this was an ornamental or naturalized occurrence (Morgan et al. 2018). Since being introduced into North America—often through ornamental means—the yellow flag iris has escaped cultivation and spread prolifically.

As yellow flag iris becomes more widespread, it will be necessary for future management to understand the mechanisms that allow the iris to spread so prolifically. There is significant literature that establishes the impacts of yellow flag iris invasions as well as literature that highlights possible control mechanisms (DiTomaso et al. 2022, Gallego-Tévar et al 2022, Jacobs et al. 2010, Spaak 2016, Tarasoff et al. 2016, Xiong et al. 2023). This literature review will demonstrate that yellow flag iris is an aggressive invasive species that has negative consequences on invaded ecosystems. While total control and eradication of yellow flag iris is not feasible because of its prolific and resilient nature, combined management techniques should be used to control and prevent further distribution and spread into vulnerable habitats. This literature review will synthesize the physical, environmental, and human-mediated mechanisms that allow the yellow flag iris (*Iris pseudacorus*) species to become invasive and will also assess possible control and habitat restoration methods.

Physical Mechanisms of Establishment

There are a few morphological characteristics of the yellow flag iris that allow it to establish in a new habitat and potentially outcompete other species. Sexual and asexual reproduction play a key role in giving the yellow flag iris its ability to rapidly proliferate. Like other semi-aquatic or aquatic plant taxa, the yellow flag iris tends to use asexual reproduction more than sexual reproduction (Gaskin et al. 2016, Johansson & Nilsson 1993). The iris utilizes underground shoot structures (rhizomes) to propagate more aboveground iris clones (Sutherland 1990).

The ability of yellow flag iris to reproduce by rhizomes is one of the key reproductive characteristics that allow it to outcompete surrounding species. Yellow flag iris rhizomes can spread widely from the original plant and can establish dense, homogenous stands (Jaca & Mkhize 2015, Sutherland 1990, Xiong et al 2023). Through flooding or other aquatic transport, rhizomes can break off and facilitate growth in other areas. Sutherland (1990) states that a stand nearly 66 feet across located in Ireland was discovered to be derived from a single rhizome fragment. Yellow flag iris rhizomes have also been found to store high quantities of carbohydrates (Tarasoff et al. 2016). This ability allows yellow flag iris to grow rapidly after the winter and to quickly grow after a rhizome fragment colonizes a new area. Additionally, the rhizome from the yellow flag iris can withstand periods of drying and was found to grow after nearly 3 months out of water (Sutherland 1990).

In addition to the propagation by rhizome, yellow flag iris forms elongate, three-chambered seed capsules after flowering that allow propagules to spread across a wider area

(Jacobs et al. 2010). There may be many seed capsules per plant, depending on the number of flowers and the amount of seeds each capsule can produce vary, however estimates range from 32 to 120 (Jacobs et al. 2010, Morgan et al. 2018, U.S Fish and Wildlife Service 2019). Seeds are also highly buoyant which allows for further water-mediated distribution (Morgan et al. 2018).

Authors also differ in their assessment of seed viability. A study performed by Gaskin et al. (2016) found that populations of yellow flag iris in the northwest were genetically different, suggesting wide-ranging seed dispersal and less propagation by rhizomes. The study also concluded that nearly 91% of seeds from the study population were viable (Gaskin et al. 2016). This indicates that high seed recruitment may play a role in the ability of yellow flag iris to establish in a wide range of areas. Conversely, Jacobs et al. (2010) reported that seeds that had been recently collected only germinated 48% of the time, while seeds treated with different techniques in a lab (scarification and rinsing) germinated 70-80 % of the time. These different reports of viability rates suggest that degree of viability varies among populations and that generalization may not be possible.

Recent studies highlight the ability of yellow flag iris seeds to survive and germinate in a variety of conditions. As indicated above, yellow flag iris can grow in a variety of aquatic and semi-aquatic conditions and can grow in both fresh water and brackish environments (Morgan et al. 2018, U.S Fish and Wildlife Service 2019). While trying to understand the limit of seed viability in more saline environments, Gillard et al. (2021) found that yellow flag iris seed were able to germinate in mildly brackish water (>25 dS/m) but did not germinate in more saline conditions. However, seeds that had been exposed to higher salinity were able to germinate after being exposed to freshwater (Gillard et al. 2021). This indicates that yellow flag iris propagules may be able to disperse through more extreme conditions and establish in further areas.

Additionally, seeds from yellow flag iris may have better germination potential in an environment with fluctuating temperatures. Yellow flag iris can be found at varying elevations and ecosystems, which already allows for tolerance of more fluctuating temperatures (Sutherland 1990). Seeds from northern California have higher germination rates when temperatures change diurnally. Furthermore, the seeds germinate under a wide range of temperature exposures (Gillard et al. 2023). This suggests that yellow flag iris can proliferate under broad conditions potentially allowing it to establish in a variety of areas.

Human Mediated Mechanisms of Establishment

Besides the innate recruitment and reproductive qualities of yellow flag iris, anthropogenic forces play a role in the introduction and encroachment of the iris around the world. Two human forces that allowed yellow flag iris to escape and become the widespread issue are the purposeful introduction through the horticultural industry as well as human-caused disturbance (Hayasaka et al. 2018, Lehan et al 2013, Maki & Galatowitsch 2004, Thomson et al. 2021).

One of the most significant reasons the yellow flag iris has established widely is its ornamental cultivation. In many areas, the iris was established after its use in water gardens and public horticulture (Xiong et al. 2023). The horticultural trade continues to be a point source for new areas of invasion of the yellow flag iris.

Although yellow flag iris already is widely distributed as an invasive species, it continues to be introduced to new areas either purposefully or accidentally via its horticultural uses. In 2004 nearly 92% of aquatic plant shipments in the United States contained either locally or federally listed noxious weeds or invasive species—including yellow flag iris (Maki &

Galatowitsch 2004). The ability of yellow flag iris to escape cultivation and propagate in a variety of conditions is concerning in the context of its continued sale and use as an ornamental.

Although purposeful introductions of ornamental species in the United States is a main pathway for the introduction of non-native species, accidental introduction also are significant (Lehan et al. 2013). Accidental introductions could include things like seed contaminants in agricultural shipments or the planting of incorrectly identified species (Lehan et al. 2013). While yellow flag iris has predominantly been purposefully introduced in regions like China, Japan, and the United States for horticulture or habitat restoration (Hayasaka et al. 2018, Xiong et al. 2023, Lehan et al. 2013), the accidental introduction pathway also may be significant in establishing new populations.

Additionally, human-caused landscape disturbance ranging from widespread agriculture to human urbanization, to infrastructure repair encourage yellow flag iris establishment and spread (Hayasaka et al. 2018, Thomson et al. 2021, Xiong et al. 2023). For example, yellow flag iris established along a portion of previously uninvaded wetland area after the reconstruction of a dam at a nearby lake. Following the replacement of the dam structure, yellow flag iris was found to be growing in the downstream wetland habitat and may have entered the area vis propagule-contaminated equipment, removed waste sediment, and inadequate pre-mitigation of small existing yellow flag iris stands before the project began (Thomson et al. 2021). Combined with its resilient growth characteristics, human-caused disturbance likely increases yellow flag iris's colonization of undisturbed areas.

It should be noted that there is limited research involving human influence on yellow flag iris invasions. However, considering the invasive qualities, widespread distribution, and the continued transport of yellow flag iris, more literature would be useful in the future to best understand factors that increase the likelihood and extent of invasion so potential management can have the best chance at reducing the impacts of yellow flag iris on aquatic ecosystems.

Consequences of Yellow Flag Iris Introductions

There may be a range of consequences following the introduction of yellow flag iris into novel ecosystems based on its ability to propagate rapidly and create dense stands (Sutherland 1990). This ability can effectively crowd out other species in the area and decrease plant diversity (Gallego-Tévar et al. 2022, Hayasaka et al. 2018, Xiong et al. 2023).

In an abandoned urban pond area in Japan there was a statistically significant decrease in species richness in sampling sites that had more than 50% iris cover (Hayasaka et al. 2018). Additionally, there were fewer native species and higher rates of other invasive species when yellow flag iris was present (Hayasaka et al. 2018). Yellow flag iris had negative impacts to plant diversity in introduced regions like California marshes (Gallego-Tévar et al. 2022). This paper demonstrates that yellow flag iris can outcompete other adjacent aquatic vegetation and decrease diversity overall in areas where it is present. This effect differs in native areas like Andalusia, Spain, where the presence of yellow flag iris has been found to increase plant diversity and richness (Gallego-Tévar et al. 2022). The absence of its native community interactions allows yellow flag iris outside of its native range to outcompete other adjacent aquatic vegetation and overall diversity.

Control Methods

Understanding physical and human-mediated mechanisms of yellow flag iris introduction as well as the consequences of its establishment is an important first step in managing this invasive species. There has been a wide range of suggested control methods to eradicate and manage the spread of yellow flag iris. Main techniques can be categorized into two groups: direct control and indirect control. Direct methods include mechanical control and herbicide use while indirect management includes biological control and the use of policy to prevent trade or enforce management practices. (Global Invasive Species Database 2023, Hulme et al. 2018, Minuti et al. 2023, Tarasoff et al. 2016).

Because yellow flag iris has a resilient shoot and rhizome structures, covering, cutting, or trampling down the yellow flag iris plants may be an effective method of direct control. However, when a benthic barrier was applied to stands of yellow flag iris for 35 days, it effectively stopped growth of the iris rhizomes with minimal regrowth after barrier removal (Tarasoff et al. 2016). Additionally, aggressive cutting of aboveground structures generated similar effects as the benthic barrier (Tarasoff et al. 2016). Trampling by cattle or other means also has proven to be an effective method at controlling the density and proliferation of yellow flag iris (Spaak 2016, Stoneburner et al. 2021).

Herbicide use is another potential control method for yellow flag iris. The Global Invasive Species Database (2023) recommends the application of glyphosate directly to stem and stalks to control stands of yellow flag iris. Similarly, the use of broadcast spraying of glyphosate, imazapyr, and triclopyr was shown to be very effective at controlling yellow flag iris around pond areas, however only the imazapyr was effective when applied at the recommended lowest concentration (DiTomaso et al. 2016). While the use of herbicide may provide wider- reaching control of yellow flag iris, there may be negative effects to all surrounding plants or other organisms due to the broad-spectrum nature of the recommended herbicides. Using herbicides labeled for aquatic use would minimize harmful effects on the surrounding environment (Global Invasive Species Database 2023).

Utilizing any means of direct control requires managers to first identify populations of yellow flag iris. Because yellow flag iris can form stands along shorelines, banks or other aquatic or semi-aquatic habitats and it has a limited flowering period, it may be difficult to survey to get precise estimates of location of smaller stands or total spread. One pre-management technique for mapping expanses of yellow flag iris is using unmanned arial vehicles (UAVs) (Hill et al. 2016). Using commercial drones to identify patches of yellow flag iris during the flowering season around two lakes in British Colombia, Canada, proved to be the most accurate method compared to standard field survey or computer imagery analysis (Hill et al. 2016). Using techniques like this could allow managers to identify populations of yellow flag iris and make the best management decisions for the area.

One method of control that lacks research is the use of biocontrol to manage yellow flag iris. There has been one study to date that utilized habitat suitability analysis to predict overlap between potential biocontrol insect predators and yellow flag iris in the southern hemisphere (Minuti et al 2023). While biocontrol may be an effective method to control yellow flag iris without use of pesticides or intensive manual removal, the differences in climactic suitability between the biocontrol agent and yellow flag iris populations may not allow for effective control. The study concluded that in the southern hemisphere, there was very little overlap between the biocontrol insect habitat and yellow flag iris habitat, however predicting areas of potential future invasion may present other biocontrol options that are better suited for the specific habitat (Minuti et al 2023).

Besides physical control of yellow flag iris, other management techniques like policy implementation may help control human-mediated movement and introduction. Because the horticultural trade is so widespread, limited monitoring of yellow flag iris distribution may play a role in unintentional introduction into vulnerable habitat. To limit the human-mediated movement of yellow flag iris in the horticultural industry, four types of import policies may be implemented (Hulme et al. 2017). First, pre-border import restrictions may assist in preventing unwanted plant material from being transported across state lines. Second, post-border bans of invasive species could allow for more stringent management and eradication of yellow flag iris. Third, industry self-regulation would allow for the horticultural industry to internally manage the spread of problematic species. Lastly, education for consumers would provide individuals with knowledge to avoid and control species like yellow flag iris in their own projects. It should be noted that when assessed by Hulme et al. (2017), there was no single policy that was able to adequately manage the movement and introduction of problematic species The integration of different aspects of each policy may ensure that species like yellow flag iris have a reduced chance of being spread by horticultural means.

Conclusions

The invasion of yellow flag iris in non-native environments is a growing concern around the world (Hayasaka et al. 2018, Gallego-Tévar et al 2022, Xiong et al. 2023). Because yellow flag iris has been transported and introduced through different human-mediated mechanisms, it has become a significant issue in both human-impacted urban areas and natural habitats alike. There are many areas that have classified the yellow flag iris as an invasive species including parts of the United States, Japan, and New Zealand, however the establishment of yellow flag iris in other areas—particularly in U.S. states like Colorado—has been relatively recent and has been minimally documented (Denslow et al. 2011). Therefore, more research is necessary to document and monitor new populations.

Due to its widespread occurrence and its resilient nature, it is unreasonable to recommend the complete eradication of yellow flag iris in non-native ecosystems. Current populations should be monitored to gain more understanding of how efficiently the plant may spread to new, vulnerable areas. Additionally, uninvaded areas, or areas of particular concern should be assessed to determine suitability for yellow flag iris to prevent further invasion. It would be feasible to directly treat smaller or newer populations of yellow flag iris rather than attempting to eradicate all populations.

Research into management of yellow flag iris has established a few main techniques to control populations both directly and indirectly. The combined use of different techniques should be investigated further to maximize efficiency and efficacy of treatments. While individual uses of manual or chemical control directly to yellow flag iris populations may be ineffective, the combined use may be the most effective approach. However, because yellow flag iris grows in many different conditions, more research should be done to assess the most effective methods for specific areas.

Because yellow flag iris was mainly introduced through human movement and can still be found in the horticultural industry (Lehan et al. 2013), implementation of policy or regulation of the horticulture industry will be extremely important to reduce the trade and accidental introduction into potentially vulnerable habitats. The most direct policy that could be implemented would be adding yellow flag iris to noxious weed lists which would require control of yellow flag iris from individual property owners and would provide guidelines from state or federal institutions on how to best control the species. Additionally, implementing bans and more stringent monitoring for yellow flag iris within the horticultural industry would reduce the unintentional transport of yellow flag iris material to wider areas.

To reduce the negative impacts that yellow flag iris has on ecosystems and to best control its spread, increasing monitoring combined with integrated management strategies will be necessary. As demonstrated above, the yellow flag iris can easily invade new areas and decrease biodiversity. More surveys should be conducted to gain the most accurate knowledge on its occurrence and how it may spread to vulnerable areas. After determining the extent of yellow flag iris in non-native areas, the most effective management techniques should be implemented to prevent more spread. Understanding the severity of yellow flag iris invasion combined with understanding how to control it is critical to making informed decisions about the establishment and management of yellow flag iris in the future.

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CHAPTER 2. GRANT PROPOSAL

Habitat Suitability Assessment and Potential Management Techniques for Yellow Flag Iris (*Iris pseudacorus*) in Boulder County Open Space, Colorado

Abstract

The management of noxious weeds is an important step in protecting biodiversity and natural spaces. One species of concern in Colorado is the yellow flag iris. This species has been cultivated for ornamental purposes but escaped cultivation to establish unmanaged populations in Boulder County. Furthermore, there is limited information on the extent of occurrence of yellow flag iris within Boulder County. The recent listing of yellow flag iris on the Colorado Noxious Weed list demonstrates that the presence of this species is concerning for Colorado ecosystems. As such, I propose a study to assess habitat suitability for yellow flag iris based on previous occurrence data. This assessment will identify similar areas in Boulder County that are at risk of yellow flag iris invasion. I will also conduct a study to compare the efficacy of manual removal and herbicide spray to control yellow flag iris. This study will provide land managers with promising management options for control of yellow flag iris. Overall, this research provides updated information on the occurrence and management of yellow flag iris in Boulder County.

Objectives

I propose to conduct a habitat analysis based on previous occurrence records of yellow flag iris (*Iris pseudacorus*) to determine the ecological conditions that facilitate the growth and proliferation of this non-native species in Boulder County. The yellow flag iris establishes and grows aggressively in aquatic and semi-aquatic habitats and has been identified as an invasive noxious weed in Colorado (List A) (Global Invasive Species Database 2023, Xiong et al. 2023). This species has been found in limited areas around Colorado including Jefferson, Boulder, and Larimer counties (Colorado Department of Agriculture 2023). Due to its current limited spread in Colorado, it is vital to conduct a habitat suitability assessment near established populations to understand where yellow flag iris already has spread and may spread in the future. I will survey adjacent areas based on ecological suitability to determine the presence or absence of yellow flag iris. Furthermore, I will identify a population of yellow flag iris to compare manual control methods and chemical control methods to understand the efficacy of each control technique.

Questions & Hypotheses

This study aims to answer two questions:

- Based on current distribution in Boulder County and what is known about yellow flag iris' habitat requirements, what is the colonization probability of yellow flag iris in surrounding habitat?
    - *Using current literature and occurrence information, similar areas within Boulder County can be assessed to determine the colonization probability and presence or absence of yellow flag iris.*

- How effective is manual control compared to chemical methods at controlling yellow flag iris in populations of yellow flag iris in Boulder County?
    - *Although it is more labor intensive, manual control of yellow flag iris at recently established populations will be more effective than chemical methods and will reduce the undesired side-effects associated with herbicides. Chemical management may provide more broad control for more established populations; however, this may have unintended consequences for surrounding vegetation.*

Anticipated Value

Yellow flag iris was recently added to the Colorado Noxious Weed List. It was placed in "List A" which includes all noxious weeds that are required to be eradicated in the state. To date, there is little information regarding the yellow flag iris invasion in Colorado besides limited population data. It is imperative to generate accurate data on its current occurrences and more importantly, to evaluate the susceptibility of un-invaded areas to prevent the weed expansion. The results of this study will provide critical information for best management practices. I intend to expand these areas of knowledge by identifying local ecological characteristics that promote yellow flag iris establishment, identifying areas in Boulder County that have high suitability for yellow flag iris invasion, and comparing manual and chemical control methods to determine the most effective treatment for Boulder County managers.

Literature Review

The yellow flag iris (*Iris pseudocarus*) is classified as an emergent aquatic perennial. The iris is often found in wetlands, marshes, along streams and lakes and can be found in brackish habitats. (U.S Fish and Wildlife Service 2019). Due to its human cultivation, yellow flag iris can also establish in urban settings along ponds or other human made infrastructure. (Hayasaka et al. 2018, Thomson et al. 2021).

The yellow flag iris displays dramatic yellow flowers on tall stems with long smooth leaves (Sutherland 1990). It reproduces sexually through seed pods and asexually by rhizomes (Sutherland 1990, Xiong et al. 2023). Rhizomes from yellow flag iris can proliferate widely from a single plant and form dense stands (Jaca & Mkhize 2015, Sutherland 1990). Individual rhizomes can break off from an original plant and be transported through aquatic means to facilitate growth in new areas (Sutherland 1990). Most seedpods can produce as many as 120 seeds each and seeds are highly buoyant, allowing for efficient water dispersal (Morgan et al. 2018, Sutherland 1990, U.S Fish and Wildlife Service 2019). These reproductive traits allow the iris to form large, dense stands that can proliferate throughout habitats and outcompete other vegetation. Furthermore, yellow flag iris is capable of growing in a wide range of conditions. The plant can grow on substrates ranging from gravel to sandy soils, can grow in a wide range of oxygen and pH levels, and has been found to grow in moderately saline environments (Jacobs et al. 2010, U.S Fish and Wildlife Service 2019). The ability of yellow flag iris to proliferate under many different conditions increases its invasion potential.

The establishment of yellow flag iris in a non-native environment can carry biodiversity consequences (Jacobs et al. 2010, U.S Fish and Wildlife Service 2019, Xiong et al. 2023). When yellow flag iris establishes a monoculture in a new environment, it can outcompete other species and cause a rapid decline in biodiversity (Xiong et al. 2023). The loss of biodiversity in a riparian or aquatic environment can further affect other bird, fish or mammal species that rely on the environment for food or habitat (DiTomaso et al. 2016).

Because of its invasive qualities, the yellow flag iris has become a species of growing concern around the world, including in the United States (Global Invasive Species Database 2023, Jacobs 2010, Xiong 2023). Yellow flag iris is a native species to Europe, Asia and north Africa but has been highly cultivated for ornamental purposes in other regions like China and North America (Global Invasive Species Database 2018, Xiong et al. 2023). Through the cultivation and human dispersal of yellow flag iris, it has become widespread and prolific in non-native ranges (Gillard et al. 2021, Jacobs et al. 2010)

One non-native range that yellow flag iris has spread to is Colorado. Sources cite differing dates of introduction and observation of yellow flag iris in Colorado. Lehan et al. (2013) indicates that yellow flag iris was introduced to Colorado as early as 1850 for ornamental purposes. Denslow et al. (2011) highlights the point that yellow flag iris was typically not accounted for in general floristic surveys and therefore may have not been properly documented. Furthermore, yellow flag iris would have been present in garden or horticultural settings and then escaped cultivation. Yellow flag iris may have been present outside of cultivation in Boulder

County in the 1970s (Denslow et al. 2011). Interestingly, the USGS records for "non-indigenous" occurrences of yellow flag iris in Colorado show that the earliest observation was not until 2011 with two occurrences in Denver and Jefferson counties (Morgan et al. 2018).

The limited information available on yellow flag iris distribution in Colorado makes it difficult to identify the full extent of its spread. Furthermore, because yellow flag iris can vigorously establish, outcompete other species, and decrease local biodiversity, it is important to detect growth early to implement eradication strategies.

Methods

Part 1. Assessment of Ecological Qualities and Habitat Survey

I will conduct an initial review of all relevant literature to determine baseline habitat requirements for yellow flag iris. I will conduct a survey of sites within Boulder County that have previous records of yellow flag iris. Occurrence data will be obtained from three sources: The Colorado Department of Agriculture, The USGS Nonindigenous Aquatic Species record database, and SEINet data portal for herbarium collections. Surveys will only be conducted in areas with the most occurrence records because of the limited time of the study period. During survey periods (April- July), data will be collected on environmental variables (Soil saturation, soil type, substrate pH, salinity, dissolved oxygen, temperature, TDS) where the yellow flag iris is present. Next, habitat variables cited in the literature will be cross referenced with survey data. Some of these habitat variables include high soil saturation, temperate conditions, and neutral to basic soil pH (Gallego-Tévar et al 2022, Sutherland 1990). After cross referencing, specific variables will be updated to include a definitive list of habitat measures that facilitate yellow flag iris growth in Boulder County.

Analysis for habitat suitability will be conducted in GIS (Geographic Information Services). The combined habitat variables from the literature and the site surveys will be mapped within the study area. Because yellow flag iris has many dispersal methods, connectivity measures such as hydrologic connectivity and human dispersal pathways (roads, trails, water infrastructure, etc.) will also be overlayed. Previous occurrence data will be overlayed. Based on the habitat variables, occurrence data, and site connectivity, other habitats at risk for invasion will be projected. Using this modelling, new site surveys will be conducted to determine the presence or absence of yellow flag iris in the at-risk areas.

Part 2. Implementation of Management Techniques

A population of yellow flag iris will be selected to be an experimental plot to test the efficacy of manual control methods and chemical control methods. The population will be selected based on ease of access and potential to minimize impacts to surrounding vegetation and wildlife. The following methodology will be similar to Tarasoff et al. (2016), Little (2013), and DiTomaso & Kyser (2016). The area will be divided into control plots, manual removal plots, and chemical spray plots. Within each plot, species richness and percent cover will be assessed before management techniques are applied. In manual removal plots, yellow flag iris will be removed by pulling, digging, or cutting above ground and below ground structures. In chemical control plots, broad spectrum herbicide (like glyphosate) will be applied directly to aboveground yellow flag iris vegetation. The Colorado Department of Agriculture (2023) and the EPA (2023) recommend the use of glyphosate (5% by volume) for noxious weeds in aquatic areas.

Assessment of yellow flag iris control will be based on percent cover of yellow flag iris that remains one year after control methods are implemented to reduce variation based on time of year and ability to identify plants outside of flowering time. Species richness will also be quantified to assess effects on non-target species. To understand longer term efficacy of this management, this study may be conducted in subsequent years.

Data Analysis Techniques

To assess the efficacy of yellow flag iris control methods, data analysis will be conducted on the species richness and percent cover data before and after treatments. All data analysis will be conducted in R. T-tests will compare the before treatment and after treatment species richness and percent cover of yellow flag iris for each treatment. Comparison between the treatment effectiveness will also be compared using a linear regression to compare across treatments.

Project Requirements

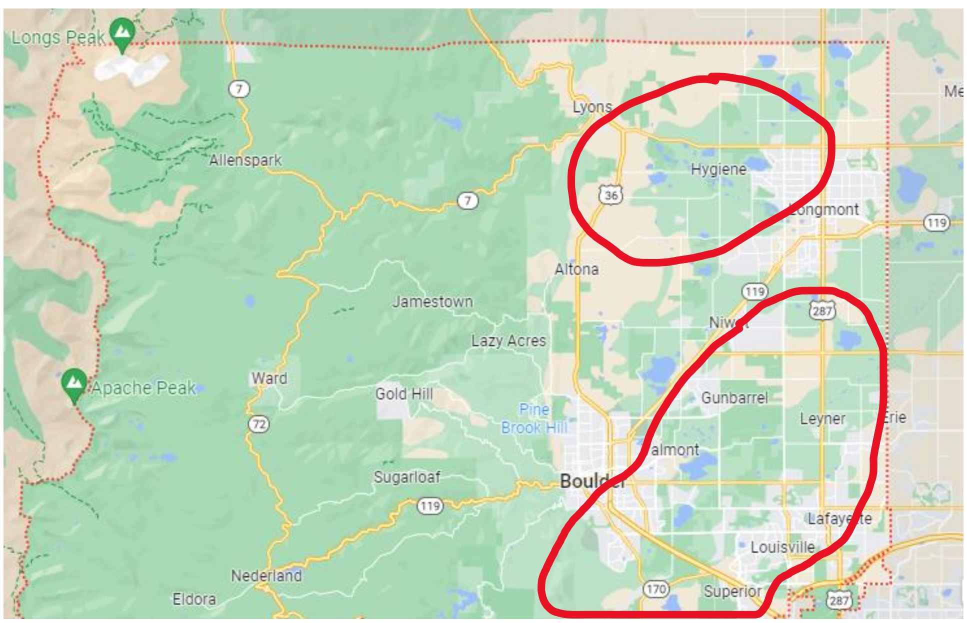
This project requires a Boulder County research permit to conduct research on county land/open space. I will also apply for an Open Space and Mountain Parks collection permit to collect vegetation samples from the research plots. These permits will be obtained no later than March 2024.

Project Schedule

Date	***Activities***	***Project Deliverable***
January- March 2024	- Conduct supplemental literature review - Apply for permits - Identify populations of yellow flag iris in Boulder County - Survey planning	Literature review for habitat variables, Sampling and collection permits
April- June 2024	- Conduct GIS analysis - Travel to site locations - Conduct site habitat variable survey - Identify focal population for management study	Site survey (during height of blooming) Identification of experimental population

June- July 2024	- Conduct manual removal and herbicide treatments - Begin compiling information for final report	Management implementation experiment Rough draft of final report
July-September 2024	- Finalize management study - Conduct data analysis - Update final report	Data analysis Updated draft of report
October- December 2024	- Finalize and submit report	Final Report

Map of potential study areas



Potential Project Impact

For the habitat suitability assessment and survey, there will be minimal impact to the focal study sites and surrounding area. The largest impact would be trampling of vegetation during survey. There may be negative impacts to surrounding vegetation at the experimental site. The manual removal of yellow flag iris may disturb surrounding vegetation and may disturb sediment. The chemical treatment has the potential to come into contact with other vegetation or runoff into the soil and water. Application measures will be taken to avoid accidental herbicide drift.

Budget

	Item	**Unit Cost**	**Total Cost**
Equipment & Office Supplies	Field supplies, documentation supplies, and asst. Equipment	$150	$150
GIS analysis student stipend	For one student to perform GIS analysis	$600 (x1)	$600
Student Field Assistant Stipend	For one student	$12/hour (x100)	$1,200
Travel	x10 round trips from Denver to Boulder sites	$0.60 /mile (x440)	$264
Researcher Stipend	For student training, fieldwork, data analysis and report writing	$3,000	$3,000
Total Request			**$5,214**

Please Note: The researcher will be donating extra supplies and time outside of the requested funds above.

Qualification of Researcher

Sarah Luper

EDUCATION

Regis University | Denver, CO Aug. 2022-Present

M.S. Environmental Biology Candidate
Anticipated graduation date- May 2024

Regis University | Denver, CO Aug. 2018- May 2022

B.S. Environmental Studies, Minor in Biology

- Dean's List awarded Fall 2020 & Spring 2021
- Biology faculty search committee member, Fall 2021
- Biology Department Mentorship program, Spring 2022

RELEVANT RESEARCH EXPERIENCE

• **Little Bear Creek Road NEPA Environmental Assessment (EA)** Fall 2023

- Worked with Clear Creek County to assess the impacts of a road improvement project on environmental resources near Idaho Springs, Colorado.

- My group and I used the NEPA process to document project impacts to the environment, developed recommended mitigation strategies, and offered alternative actions that would minimize effects to the environment.

• **Impacts of Indaziflam Application on Front Range Grasslands** Fall 2022- Spring 2023

- Collaborated with Denver Mountain Parks to assess the impacts of the graminoid-controlling herbicide indaziflam in three Front Range parks.

- Used plant and soil quadrat sampling to quantify effects of the herbicide spray on plant species composition, soil biota, and the additive impact of bison grazing.

- Results presented at the 2023 CSU Front Range Student's Symposium and Denver Mountain Parks.

• **Coal Creek Aquatic Integrity Research** Spring 2023

- To understand wildfire effects on stream biological integrity, my team analyzed physical habitat characteristics and aquatic macroinvertebrates in Coal Creek before and after the Marshall Fire.

- Collected and identified aquatic macroinvertebrates, water chemistry data, and riparian habitat data to understand how wildfire affected Coal Creek.

- Results presented to the City of Louisville in April 2023.

• **Fire Potential in Rocky Mountain National Park** Spring 2022

- To understand future wildfire probability, I researched past fire history and influential fire factors in Rocky Mountain National Park.

- *Utilized GIS and spatial datasets to identify and map both contributing factors and historical wildfire areas to project possible future fire areas.*

WORK EXPERIENCE

Invasive Species Mapping Intern | Denver Botanic Gardens May 2023-Present
Denver, CO | Mon- Fri | 40 hours/week
- *I took initial population and reproduction data on potentially invasive horticultural species at Denver Botanic Gardens. Identified specific species, took field data, and accurately entered large quantities of data to be used for future analysis.*

Office Receptionist | Echter's Nursery & Garden Center May 2022-Aug. 2022
Wheat Ridge, CO| Mon- Fri | 40 hours/week
- *I was responsible for answering customer phone calls, providing general plant information, handling business mail, maintaining the office space, and designing and building store signage with Adobe InDesign.*

Cashier & Sales Associate | Echter's Nursery & Garden Center May 2019- Aug. 2021
Wheat Ridge, CO| Schedule variable | 40 hours/week
- *In addition to performing customer service duties and training new department hires, I assisted a wide range of customers with answering landscape and garden questions, identified ornamental and native landscape plants, and provided expertise on plant care.*

VOLUNTEER EXPERIENCE

Teacher's Assistant | Compass Montessori Jan. 2017- May 2018
Golden, CO
- *I graded biology assignments, independently cleaned/maintained lab space, set up and prepared laboratory activities, and reared Axolotls from egg to adult for genetics lesson.*

Service-Learning Project | Compass Montessori May 2018
Golden, CO
- *Volunteered to independently organize aquatic habitat restoration, identified and removed invasive species, tested, and maintained water quality, reconstructed small dammed area. I presented my progress to teachers and high school class.*

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CHAPTER 3. JOURNAL MANUSCRIPT

Effects of Trails and Invasive Grass *Bromus inermis* on Species Richness and Community Composition at Two Ponds National Wildlife Refuge

Abstract

Recreational trails and the presence of invasive species such as *Bromus inermis* can both disturb grassland community composition. Trails can act as dispersal corridors for plant material, but can also be a source of trampling and compaction. Invasive grasses like *Bromus inermis* can easily establish in grasslands and decrease species richness by increasing competition. Understanding these effects, this study aims to understand how both the presence of recreational trails as well as the presence of *Bromus inermis* affect species richness and overall community composition at Two Ponds National Wildlife Refuge. Belt transect sampling took place in fall 2019 and again in 2021. I used generalized linear modelling and mixed effect modelling to understand how distance from trails affected species richness and percent cover of *Bromus inermis*. I used non-metric multidimensional scaling (NMDS) to assess overall species community composition. I found that species richness was negatively impacted by distance from trail while percent cover of *Bromus inermis* was positively impacted. Additionally, species composition varied according to distance from trail, with differing effects for individual species. This study concludes that walking trails may act as a dispersal corridor for species as indicated by higher species richness while trails may hinder the growth of *Bromus inermis* due to trampling and soil compaction. Furthermore, the variation in community composition may result from individual species being able to disperse and propagate more easily along the trails. My

findings contribute to ongoing research into management and ecology of urban grasslands in the face of several anthropogenic disturbances.

Introduction

Disturbances affecting grasslands has been a topic of continuing interest and concern for both land managers and scientists for many years (Belsky 1992, Collins & Barber 1986, Geiger & McPherson 2005.). Grasslands are prone to several disturbances including fire, livestock grazing, non-native plant invasions, and creation of recreational trails through the ecosystem (Belsky 1992, Hobbs & Huenneke 1992, Jordan 2000). Larger, more natural grasslands as well as urban grasslands are also impacted by these disturbances—of particular interest are the impacts of recreational trails and invasive plant invasions. These disturbances often have differing impacts on the plant community composition and diversity in grassland ecosystems.

Recreational trails provide access to diverse natural areas for a variety of activities such as hiking, cycling, wildlife viewing, and horseback riding (Jordan 2000). While trails have many beneficial uses for human recreation, recreational trails in grassland ecosystems have been shown to have varying effects on the surrounding plant community. Trails often influence the movement of plant species by acting as transportation corridors for seeds and other plant material to be moved by either human or animal movement (Benninger-Truax et al. 1992). At the edge of trails, greater species diversity has been shown to occur (Root-Bernstein & Svenning 2018), although in many cases trails were shown to significantly decrease diversity and alter community composition compared to habitat further away from the trail's edge (Ballantyne & Pickering 2015, Crisfield et al. 2012, Queiroz et al. 2014). Furthermore, intensity of trail use may increase these effects (Potito & Beatty 2005, Wolf & Croft 2014).

Trails also impact both plant material and soils through trampling and compaction (Crisfield et al. 2012). While trampling occurs in a limited area around trails, it may harm less-resilient plants along the trail edge (Jordan 2000). Likewise, substrate compaction can also harm plants close to trails by reducing oxygen and air movement through the soil making it difficult to form root structures and establish propagules (Chisholm & McCune 2024, Wolf & Croft 2014). These direct disturbances caused by trails may allow for more disturbance-tolerant or invasive species to establish closer to the trails and potentially outcompete less resilient species.

While recreational trails can directly influence the movement and composition of grassland flora, disturbance can also facilitate invasive and non-native species establishment in grasslands which can lead to a myriad of effects on the natural ecosystem. In instances with previous disturbance like fire, an introduction of an invasive grass species decreased the resilience of native species to recolonize after the disturbance (Flory & Clay 2010). Similarly, when humans introduce a new plant species into a landscape, it could lead to that species benefitting from disturbance and altering community structure (Meyer et al. 2021). Overall, the introduction or movement of an invasive plant species into a grassland system can alter local species diversity, increase competition, and potentially reduce resource availability (Fink & Wilson 2011, Gabbard & Fowler 2007).

One such invasive species prevalent in grassland ecosystems is *Bromus inermis*, or smooth brome. This grass species was introduced to the United States in the late 1800s to be used as livestock forage but has since proliferated into other ecosystems (Dillemuth et al. 2009, Fink & Wilson 2011). The cool season graminoid often establishes in nutrient rich areas and will form dense root systems that allow the grass to uptake nutrients prolifically and outcompete other native species (Ellis-Felege et al. 2013, Fink & Wilson 2011, Stotz et al. 2017). The ability of *Bromus inermis* to quickly establish and form a dense stand enables it to negatively affect species diversity to alter community composition in grasslands (Palit & DeKeyser 2022).

While we understand the general impacts of both recreational trails and invasive species establishment in grasslands, less research has been conducted into how both trail presence and invasive species presence can affect community composition and species richness in an urban grassland. This study addresses how the presence of walking trails and the non-native grass *Bromus inermis* affect the community composition and species richness of grassland plants at Two Ponds National Wildlife Refuge (TPNWR). Because trails can directly disturb plant species and soil by compaction, trampling, and propagule distribution, community composition of plants at TPNWR will change based on differing proximity to the trails. If this first hypothesis is supported, I predict that there should be a differing plant community structure closer to the trail compared to farther away. Further, I predict that there will be a positive relationship between species richness and increasing distance from the trail's edge compared to farther away. Second, because trails can act as transportation corridors for plant material, there may be a difference in *Bromus inermis* cover close to the trail compared to farther away. Specifically, I predict that there will be higher percent cover of *Bromus inermis* closest to the trail compared to farther away. Lastly, because two sampling efforts were undertaken in different years, I hypothesize that there will be a difference in results between year. Because two years passed between sampling efforts, there may have been time for invasive or weedy plants like *Bromus inermis* to establish more broadly and outcompete other species. I predict that there will be higher presence and percent cover of *Bromus inermis* during the second sampling period compared to the first. This study highlights the importance of understanding how recreational trails may affect grassland plant community composition and the establishment of invasive plants while providing insight into potential management implications.

Methods

Site Description

Two Ponds National Wildlife Refuge (TPNWR) is a protected area managed by the U.S Fish and Wildlife Service. It is located northwest of Denver, Colorado and contains approximately 72 acres of grassland and other riparian and woodland habitat to support a variety of plant and wildlife. The area is divided by a canal into a larger recreational area to the west and a smaller education and recreation area to the east. Both sections have crushed rock and hardpack dirt walking trails, while the smaller area that contains three small ponds. We collected data for this study in the larger, western portion of TPNWR.

Field Methods

Data collection occurred in Fall of 2019 and again in Fall of 2021. Between twenty-eight and thirty-one GPS points were created randomly along the trails for the sampling sites. At each site, a 26 meter transect was placed perpendicular to the trail. Starting at the edge of the trail, 1m x 1m quadrats were placed every other meter along the transect. For each quadrat, we identified all plant species, percent covers of each, and percent cover of bare ground and leaf litter.

Analytical Methods

First, to quantify the effect of distance from trail's edge on species richness within quadrats I used a Poisson distributed generalized linear model (GLM) and a generalized mixed effect model (GLMM) on the pooled data from 2019 and 2021. I first fit GLMs with richness as a function of co-predictors distance and year to identify the fixed effects that would need to be used in the best model. I then fit GLMMs with transect number as a random effect on the intercept or on both the intercept and the effect of distance. After fitting this suite of models, I assessed which model best fit the data by comparing AIC scores with a significance cutoff of 3.0. To quantify the effect of trails on percent cover of *Bromus inermis*, I used the same analytical methods with the percent cover of *Bromus inermis* as the response variable and using binomial distribution to account for proportion data.

Second, to quantify the influences on species composition at TPNWR I performed non-metric multidimensional scaling (NMDS) with the pooled data from 2019 and 2021. This analysis used the metaMDS function from the vegan package in R (Oksanen et al. 2022, R Core Team 2022). I calculated the Bray-Curtis distance metric from the percent cover data for plant species and ran the function for 2,000 iterations with two dimensions. After running the model, I projected distance from trail variable onto the ordination to quantify the strength of correlation between distance from trail and plant community structure along NMDS axes 1 and 2 using the envfit command in vegan (Oksanen et al. 2022). I fit linear models to understand the effect of distance from trail on community composition as quantified by scores on each of the two NMDS axes.

Results

Species Richness

In 2019, there were a total of 29 unique species and in 2021 we found 22 species among all transects at Two Ponds National Wildlife Refuge. I found that increasing trail distance had a negative effect on species richness. After creating fixed effect generalized linear models with co-predictor of year, the best model included only distance as a fixed effect and excluded year. The next best model included year as an additional co-predictor and was a comparably good fit compared to the previous model (ΔAICc 0.13).

After fitting mixed effect models without the co-predictor of year, I found that the Poisson-distributed mixed effect model with species richness as a function of distance and a random effect of transect number on the intercept only was the best model. The next best model included random effects of distance on the intercept and on distance but was not a comparably good fit compared to the previous model (ΔAICc score: 12.89). After accounting for transect number, with each meter away from trail there was a significant 1.11% decrease in species richness (95% CI: 0.03% to 1.86%, z-stat: -2.825, p-value: 0.005, Figure 1).

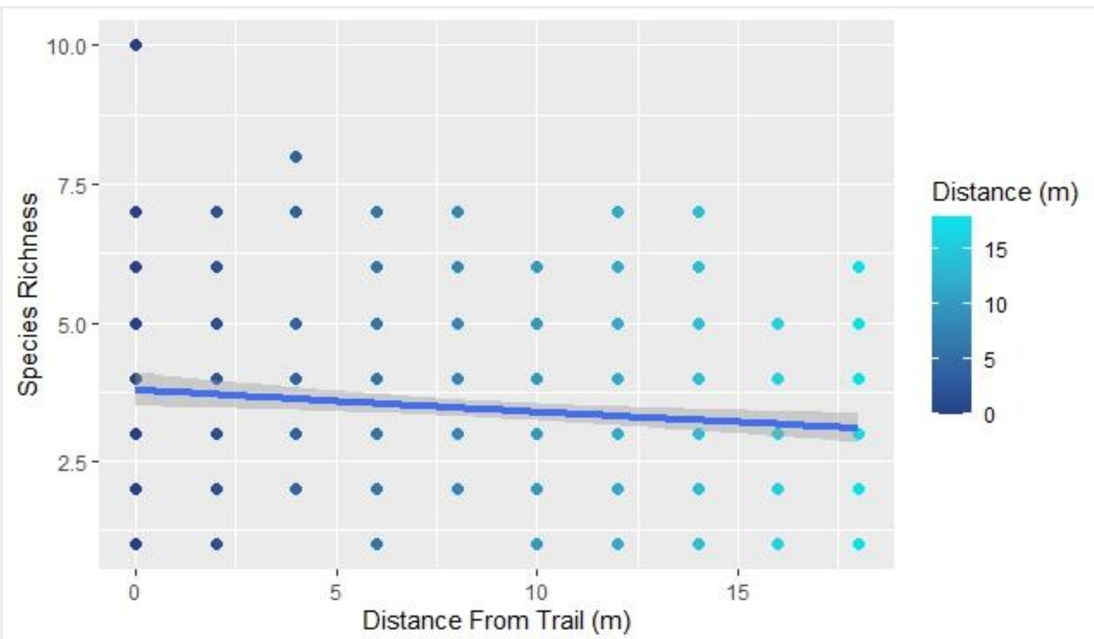

Figure 1. Fewer plant species were found farther away from walking trails. Points represent individual quadrats; blue line represents the best fit negative relationship between trail distance and species richness and corresponding shading shows 95% confidence interval.

Percent Cover of Bromus inermis

I determined that percent cover of *Bromus inermis* increased further away from the trail's edge. After creating fixed effect generalized linear models with co-predictor of year, the best model only included *Bromus inermis* percent cover as a function of distance only. The next best model included year as a co-predictor and was a comparably good fit compared to the previous model (ΔAICc: 0.61) so the model was not used. I found that a mixed effect model with the proportion of *Bromus inermis* as a function of distance with random effects on intercept and distance was the most appropriate model. The next best model included random effects of distance on the intercept and on distance but was not a comparably good fit compared to the previous model (ΔAICc score: 7.32). After accounting for transect number there was a significant 10.1% increase in the odds of finding *Bromus inermis* for each meter further from the trail (95% C.I.: 3% to 17.3% increase in odds, z-stat: 2.981, p-value: 0.003, Figure 2).

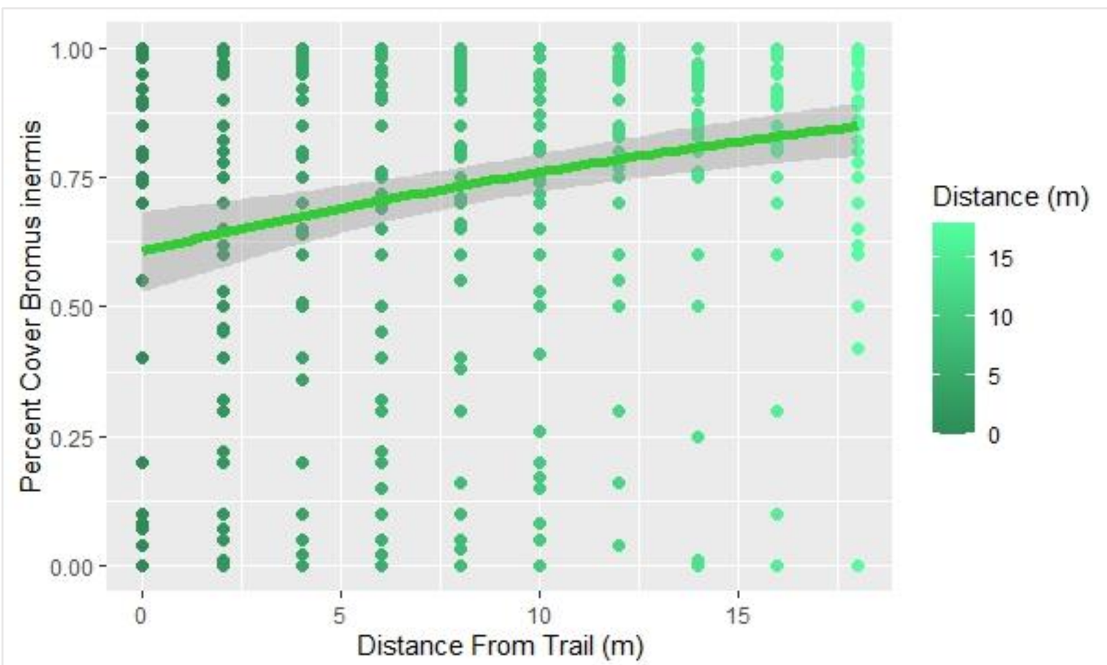

Figure 2. *Bromus inermis* percent cover increased away from trail's edge. Points represent individual quadrats; green line represents the best fit positive relationship between trail distance and *Bromus inermis* cover, and colored shading shows 95% confidence interval.

Plant Community Composition

The NMDS performed on the plant community data failed to converge after 2,000 iterations; however, the analysis reached a low stress value of 0.086, indicating a relatively good fit of the ordination. The first NMDS axis accounted for 82.2 % of the variation in the model while the second NMDS axis accounted for only 15.5 % of the variation. After projecting distance onto both NMDS axes, I found that there was a strong positive association between distance and the first NMDS axis (r = 0.987, p-value: >0.001) and a weaker association between distance and the second NMDS axis (r = -0.160 p-value: >0.001) (Figure 3).

When plotted, *Bromus inermis* had a positive species score on the first NMDS axis (0.2707) with a negative species score on the second axis (-0.0158). Other species with scores similar to *Bromus inermis* included: *Panicum capillare* (NMDS 1: 0.39303, NMDS 2: -0.05866), *Bassia scoparia* (NMDS 1: 0.42577, NMDS 2: -0.05694), *Symphyotrichum falcatum* (NMDS 1: 0.31666, NMDS 2: -0.18301, and *Chenopodium berlandieri* (NMDS 1: 0.27656, NMDS 2: -0.12672). Species loading on the NMDS axes opposite *Bromus inermis* include: *Rosa woodsii* (NMDS 1: -0.59495, NMDS2: 0.71100), *Lactuca serriola* (NMDS 1: -0.89512, NMDS 2: 0.17268, and *Tragopogon dubius* (NMDS 1: -1.23825, NMDS 2: 0.67976). There was a broad variation in species loadings onto the NMDS 2 axis. Species like *Bromus tectorum* (NMDS 2: 1.69256) and *Verbena bracteata* (NMDS2: 1.44060) loaded positively, while *Rumex crispus* (NMDS 2: -2.06575) and *Poa pratensis* (NMDS 2: -1.61560) had the most negative scores.

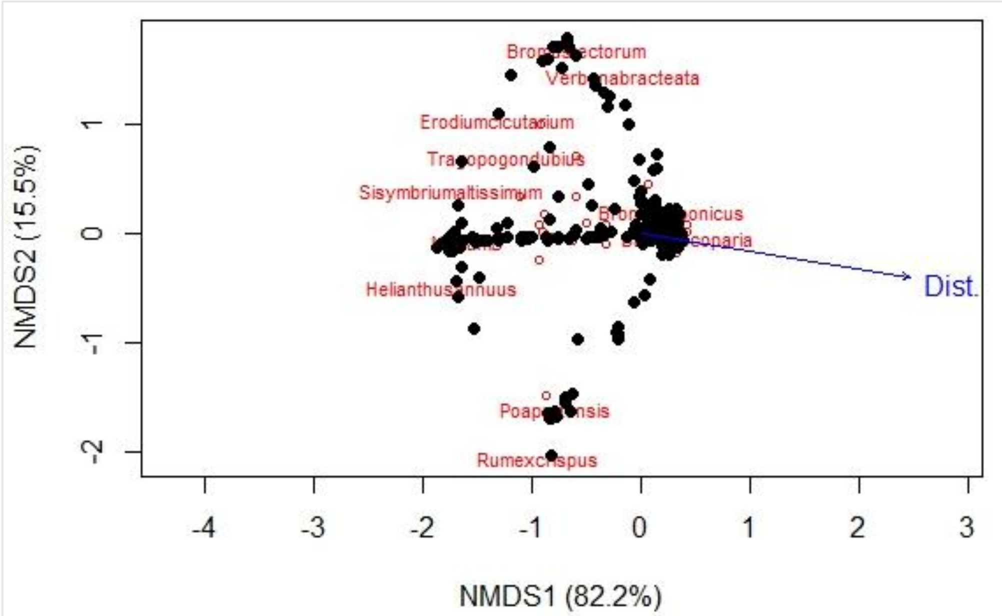

Figure 3. The plant community at Two Ponds National Wildlife Refuge strongly varied according to distance from trail. The blue arrow represents the strength and direction of the distance variable that correlates with plant community structure. Red names represent species scores along the axes, and black points represent site scores.

Discussion

This study investigated the effects of disturbance by recreational trails and the presence of the invasive grass *Bromus inermis* at Two Pond National Wildlife Refuge. To determine the effects of trail and to understand community composition, I collected percent cover and species richness data from the grassland plant communities at TPNWR between 2019 and 2021. I found that trail distance negatively affected species richness (Figure 1), and positively affected percent cover of *Bromus inermis* (Figure 2). I further determined that distance had a broad influence on community composition using NMDS (Figure 3). My results show recreational trails and the presence of *Bromus inermis* at Two Ponds National Wildlife Refuge have differing effects on the species richness and community composition overall.

Impacts to Species Richness

Contrary to my first predictions, I found that there was higher species richness closer to trails compared to areas farther away. I hypothesized that because trails are a source of trampling and compaction, less resilient species would not be able to grow along the trail edges resulting in lower species richness (Crisfield et al. 2012, Jordan 2000). These predictions aligned with other findings that demonstrated negative impacts of trails to keystone plant species (Ballantyne & Pickering 2015). Because TPNWR is a popular recreation area for the surrounding neighborhood community, species richness could be higher near trails due to the trail acting as a dispersal corridor instead. When species are disturbed by passing humans or animals, propagules or other plant material may stick to or otherwise be transported to new areas along the trailside (Pickering & Mount 2010). My results align with those found by Benninger-Truax et al (1992) who found that there were higher numbers of species along trails compared to interior forest in Rocky Mountain National Park. Through a meta-analysis, Root-Bernstein & Svenning (2018) found trails created by human foot traffic had positive influences on species richness and diversity in natural areas where trails were created. Conversely, lower species richness farther away from the trail could be the result of a combination of decreased human-mediated dispersal and higher competition between other species like *Bromus inermis* which will be discussed below. Despite trails being a disturbance, they may be promoting higher species richness and enabling species to proliferate more effectively.

Impacts to Bromus inermis *Cover*

In contrast to my second predictions, I found higher percent cover of *Bromus inermis* farther away from trails compared to closer. Because *Bromus inermis* is a highly competitive and invasive species, the increase in percent *Bromus inermis* cover away from trails may have been due to the aggressive growth habit that allowed it to establish higher biomass and easily outcompete other species (Dillemuth et al. 2009). There is significant literature that finds *Bromus inermis* to be a diversity limiting species (Dillemuth et al. 2009, Palit & DeKeyser 2022, Flory &

Clay 2010, Fink & Wilson 2011). *Bromus* has been found to cause considerable resource strain and can reduce water and light availability therefore making it difficult for other species to establish (Fink & Wilson 2011). Like findings by Krebstein et al. (2014), the disturbance by trampling and soil compaction may be reducing the belowground growth of *B. inermis* as well as aboveground growth allowing the persistence of other species closer to the trail.

Impacts to Grassland Plant Community Composition

Plant community composition at TPNWR was dominated by *Bromus inermis*. The high percentage of community variation explained by the first NMDS axis (82.2%) and the minimal variation explained by the second NMDS axis (15.5 %) indicates that there is low community variation overall. Additionally, no convergence of the NMDS model further explains the lack of variation. Interestingly, there was low stress after running the analysis, which indicates there may be an ideal convergence, however the NMDS failed to find a single optimal solution.

While there is a dominance of *Bromus inermis* in the fields at TPNWR, trails are an influential factor in shaping community composition. Some species were highly influenced by the distance variable like *Bromus inermis* such as *Convolvulus arvensis*, *Ambrosia psilostachya*, *Bromus japonicus*. It is interesting to note that these species are all relatively aggressive-growing (Bogardus n.d., Colorado Department of Agriculture n.d., Howard 1994). However, because these species scores were closely related to *Bromus inermis* they may also have difficulty establishing near trails and instead have more success away from the disturbance. On the other hand, species like *Pascopyrum smithii*, *Helianthus annuus,* and *Sisymbrium altissimum* had species scores more associated with trails. For example, *Pascopyrum smithii* is a native grass species capable of living in disturbed areas and is often used for erosion control and revegetation (USDA 2002). Likewise, *Helianthus annuus* is a native species that is often found near roadsides and can establish in a variety of conditions (USDA 2000). The presence of hardy species found closer to trails could indicate that these species could be easily established by trails and contribute to higher diversity along trails. Continued analysis of different diversity indicators like community evenness and considering native/non-native plant status could further differentiate effects on community composition.

Conclusions

At Two Ponds National Wildlife Refuge, trails positively influence species richness and the invasive grass *Bromus inermis* dominates in this community. Both trails and the presence of an invasive grass are disturbances to grasslands and can have different effects on the overall community composition. While trails act as dispersion corridors for plant species, invasive plants like *Bromus inermis* create higher competition and thusly lower species richness overall. This study demonstrates the importance of understanding how both recreational trails and the presence of *Bromus inermis* can influence community composition. Further, because Two Ponds National Wildlife Refuge is an area used for wildlife conservation, education, and recreation, it will be important to determine how to best balance the impacts caused by trails and invasive species. If it the goal of land managers at TPNWR to restore or increase species diversity, or native species richness, continued research into these dynamics would inform best management practices for the future. Similar work could also benefit other grassland sites experiencing a combination of anthropogenic disturbances from recreation and invasive plants.

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CHAPTER 4. STAKEHOLDER ANALYSIS

Environmental Stakeholder Analysis: Use of Species Watchlist to Monitor and Manage Potentially Problematic Plant Species at Denver Botanic Gardens

Introduction

Botanic gardens have a history of providing a wide variety of resources to a broad community (Chen & Sun 2018, Williams et al. 2015). Botanic gardens act as centers for scientific education, community outreach, and living botanical collections (Ballantyne et al. 2008, Donaldson 2009, Gaio-Oliveira et al. 2017). These collections of plants can contain hundreds of species from all regions around the globe and include all types of taxa (Chen & Sun 2018). Plant collections in botanic gardens can act as areas of high biological diversity, and promote wider ecological research and horticultural inspiration for visitors. However, when species from diverse regions are brought to a new environment, it is unknown how some species may grow or reproduce in the area. This can lead to some species unwantedly proliferating widely or escaping their cultivation into other areas. If such species also escape into the wider environment, there could be significant consequences for the outside ecosystem like the establishment of an invasive species. One institution that possesses the resources to tackle this issue in the beginning stages is Denver Botanic Gardens. This botanic garden incorporates living collections, research, and education which all play a role in understanding plant dynamics. I recommend that it would be most beneficial to establish a watchlist of potentially problematic species and monitor their reproduction and spread within the boundaries of Denver Botanic Gardens. Additionally, I recommend using this data to assess whether a species should be managed or removed from the collection if it poses a problem or cannot be controlled easily. While research and management of species does take time and resources, early understanding and prevention of plant escape could minimize the time and resources used compared to those used to manage a new invasive species.

Background

Humans have been transporting and establishing non-native species into new regions and environments for many uses (Reichard & White 2001a). Agriculture has been a common reason for introductions of new species; however, many other species have been introduced for horticultural uses (Marco et al. 2010). Botanic gardens have been an important component of collecting, researching, and distributing new species for food, agriculture, and ornamental purposes, however the shift to procure species for historical garden trends has also influenced the types of species being transported (van Kleunen et al. 2018). Historically, horticulture and the establishment of botanic gardens stemmed from the desire to cultivate more utilitarian species in a broad range of areas (van Kleunen et al. 2018). During the 19th and 20th centuries, “plant hunting” was extremely popular throughout Europe and the United States bringing new species from around the world to these regions for ornamental horticulture (Reichard & White 2001b). Plants procured from other regions has continued to be a pathway that botanic gardens use to introduce new species to their collections (Reichard & White 2001b).

With the increase of global horticulture trade, the movement of species outside of their original ranges is common (Padayachee et al. 2017, van Kleunen et al. 2018). When plants are transported from their native ranges to new environments, it is sometimes unknown how the species will respond. It is estimated that more than half of all vascular plant species are included in 185 public and botanic gardens around the world (van Kleunen et al. 2018). With the sheer number of species represented, it is not unlikely that a small portion may become problematic in the areas they are transported to. In this case, invasive species or problematic species are broadly defined as a plant species that is not native to a particular region and through introduction can cause negative impacts economically, environmentally, or to human health (Emanuel et al. 2011).

A significant portion of non-native species used in the North American horticultural trade and those found in botanic gardens are not native (Reichard & White 2001b). Further, most invasive or noxious species globally have been introduced through ornamental horticulture (Bell et al. 2003, Hulme et al., 2018, van Kleunen et al. 2018). For example, the yellow flag iris (*iris pseudacorus*) has been widely used throughout North America, Japan, China, South Africa, and Australia as an ornamental and restoration species (Global Invasive Species Database 2018), however it has escaped cultivation to become an invasive aquatic species in all these areas.

Interestingly, horticultural practices have tended to select for species that possess qualities that could make them problematic or invasive (Bell et al. 2003, van Kleunen et al. 2018). When selecting species to cultivate for the garden, some traits like ease of growing, wide ecological amplitude, and pest resistance make some species popular for horticulture and botanic gardens (Hulme 2011). Additionally, fast growth or high seed production is also a desirable trait in some ornamental plants, however these qualities can also contribute to unintentional escape into wider environments (Bell et al. 2003, Hulme 2011).

There are many negative consequences when non-native species escape into natural ecosystems. If a species can proliferate outside of human cultivation, larger ecological issues may arise. Invasive plants can reduce native plant diversity and abundance and increase competition for resources that can further degrade other environmental resources (Lehan et al.

2013). Further, management of new and established populations takes enormous resources. For example, in Oregon it costs more than 125 million dollars per year to manage noxious weeds (Emanuel et al. 2011). Likewise, in agricultural settings, billions of dollars per year are spent managing noxious weeds in the United States (Lehan et al. 2013). Economic and ecological impacts are points of concern among many stakeholders, making tracking and management imperative for institutions like botanic gardens.

Denver Botanic Gardens (DBG) is a non-profit botanic garden in the heart of Denver, Colorado. Spanning 24 acres, DBG hosts a large variety of garden types ranging from native Colorado collections, to traditional Japanese styled gardens, to a large tropical conservatory (Denver Botanic Gardens n.d.). Denver Botanic Gardens often curates plants and herbarium collections from areas of similar climate to Colorado like high-altitude alpine regions and windy steppe regions (Denver Botanic Gardens 2021). These climate regions often support species that will also grow successfully in Colorado gardens and some species could be introduced to the wider horticultural market (Denver Botanic Gardens 2021). Current estimates indicate that DBG has more than 50,000 documented plants in the gardens (Denver Botanic Gardens 2021). DBG indicates that many of the plants introduced into the collections are acquired from a variety of sources such as wild collection, multi-organizational specimen exchanges, and from garden centers and nurseries (Denver Botanic Gardens n.d).

One program focused on introducing new and successful species to the Colorado and surrounding regions is Plant Select. This collaboration between Denver Botanic Gardens and Colorado State University aims to provide a range of horticultural plants that not only have desirable visual qualities but also have attributes such as drought resistance, wide habitat suitability, and resistance to pests and disease (Plant Select n.d.). Like Denver Botanic Gardens, the Plant Select program often curates species from a variety of sources, and plants are trialed to assess their growth and reproduction. If species are found to possess the desired qualities and deemed to grow well in the region, Plant Select will introduce the plants to the broader horticultural industry and Denver Botanic Gardens will add it to their collections (Denver Botanic Gardens n.d.).

In addition to the Plant Select program, Denver Botanic Gardens also highlights ongoing programs including plant conservation research, native gardening education, and the biannual plant sales. Through their "Living Collections Strategy," DBG has policy to ensure that there is no admittance or distribution of federal or state listed noxious weeds within the gardens and species will be eradicated appropriately. Further, the Gardens will not distribute known invasive plant material except for specific research purposes (Denver Botanic Gardens 2021). While these policies ensure that known invasive species are not being disseminated by the DBG, there is somewhat vague policy in place for potentially problematic species that are not yet considered invasive.

For Denver Botanic Gardens, there is a conflict between the mission of the gardens to preserve and collect species for conservation purposes and the potential for species to escape or become problematic over time. There is cost and a time limitation associated with managing problematic species if they occur. Additionally, there could be negative economic and reputational repercussions for the larger horticultural trade if species are found to be problematic. Overall, this is a conflict among the interests of diversity in botanical collections, business, protection of the environment, and the desire and need for humans to move plants.

Stakeholders

Horticulturalists

Horticulturalists at Denver Botanic Gardens have direct influence on species entering and growing at the gardens. Because there is a diverse array of garden types at DBG, it is the responsibility of each horticulturalist to manage their gardens. Horticulturalists also have direct knowledge of particular species and may provide anecdotal information about certain species that could be posing an issue in their garden beds. Many horticulturalists value plant diversity and conservation while maintaining aesthetics within the garden's collections. Additionally, horticulturalists often value the introduction of new species that are regionally appropriate and horticulturally interesting (Reichard & White 2001b).

Horticulturalists may have differing opinions on the management of potentially problematic species. For example, some could argue that it is the responsibility of the botanic garden to manage and inform about species in the collection that could pose a threat. Other horticulturalists may see that it is limiting the garden collections to only contain native or non-invasive species (Reichard & White 2001b) and it is the responsibility of the botanic garden to promote high diversity in the collections.

Horticulture Business Owners

Current horticulture businesses are generally categorized into smaller local nurseries that service local regions, or larger businesses that provide wider reaching distribution (Reichard & White 2001b). Business owners will often value maintaining profits and keeping customers interested with new and diverse plants that will inspire customers to return and to make purchases. Because much of the horticulture industry relies on plants that perform well or have desirable qualities like easy growth habit or large seed production (Bell et al. 2003), horticulture businesses like seed suppliers, local nurseries, and wholesale growing productions would be heavily impacted if a species was determined to be problematic or invasive. Businesses could lose revenue and could also have to use time and resources to eliminate a particular species from their stock.

Denver Botanic Gardens Visitors

There is growing evidence that botanic garden visitors gain a positive environmental outlook after visiting a botanic garden (Williams et al. 2015). They will also most likely gain design inspiration for their own gardens. Visitors rely on botanic gardens to provide up to date information and gardening knowledge. If a species is included at a botanic garden that is at risk for escape from cultivation, visitors may be encouraged to try and plant the species themselves, creating a larger escape risk into the community.

Surrounding Community (Homeowners)

Homeowners in the community surrounding Denver Botanic Gardens can gain access to horticultural knowledge, inspiration, visual quality. People who have houses close to the gardens value a community resource close to their home that provides landscaping inspiration and garden design for their own homes. Homeowners rely on the garden to manage weedy species and not allow them to enter their own garden spaces. Like botanic garden visitors, including problematic species in the living collection may inspire homeowners to include the species in their own gardens, creating a larger distribution and management issue.

Surrounding Ecological Community (including parks)

The ecological community—who may include conservation groups, land managers focused on conservation, and other advocates for responsible ecosystem management—value ecosystem services and the continued existence of native plant communities. The escape of problematic or weedy species may have negative consequences like decreased species diversity and increased competition between species for resources (Lehan et al. 2013). For example, Cheeseman Park directly joins Denver Botanic Gardens, and escaped species could cost managers time and resources to manage possible spread of species further into the park.

Development Department. / Philanthropy Department

DBG's development department or philanthropy department relies on visitor donations and funding. Being a non-profit organization, Denver Botanic Gardens strongly benefits from donations and visitor support. Maintaining donations relies on satisfaction of the visitors. If donors do not see their favorite plants cultivated or included in the botanic garden's collections, they may be less likely to continue to give their financial support. Donor satisfaction, and therefore donations themselves may be negatively impacted if visitors are concerned about their favorite species or learn that species they have seen there before are potentially problematic. Furthermore, it may be detrimental to donations if potential donors or visitors are exposed to negative press if problematic species were to escape.

Recommendations & Conclusions

Based on the values of all stakeholders, I emphasize the use of a horticultural species watchlist to monitor and track species that pose potential issues within the gardens or have the potential to escape cultivation into the surrounding environment. Creating a watchlist would allow horticulturalists to utilize potential anecdotal data on specific species and use it as a starting point for monitoring. Beginning the process to monitor and track species as soon as concerns arise allows for a rapid response and faster management action if the species demonstrated invasive or problematic qualities.

Monitoring species over the course of multiple growing seasons would be necessary to assess reproduction and spread of the species. For example, if a population of an ornamental grass proliferated over a few growing seasons into distant garden beds where it was not intentionally planted, this may be a cause for concern and the species could be added to the watchlist. Similarly, if a species had an aggressive growth habit and propagules crowded out other intentionally planted species, this might also be reason to add it to a watchlist. This process would be particularly applicable to the Plant Select program though Denver Botanic Gardens because their trial process could also assess for invasive or weedy qualities before introducing the plants to the wider horticultural industry.

This solution would indeed take time and resources from horticulturalists within Denver Botanic Gardens, however once a monitoring system was in place, there less resources would have to be allocated to the project. Additionally, a watchlist would be the least impactful for horticultural businesses because they could be made aware of potentially problematic species without limiting their availability of a product. Furthermore, a watchlist would allow for heightened vigilance of specific species within the Denver Botanic Gardens and more management could occur to limit possible escape into the wider community. Adding species to a watchlist would not limit their growth in the gardens, however if more management were necessary, propagules could be stored for research and further education without having the species actively growing in the living collection.

Future work could include creating an analysis tool to assess risk for species. Similar species risk assessment tools exist. For example, a weed risk assessment (WRA) developed for Australia has been a widely used approach that incorporates a broad range of questions that look to assess risk of species becoming invasive (Hulme 2012, Pheloung et al. 1999). However, utilizing other region's weed risk assessments may not be the best course of action because there may be other factors to consider like difference of environment or difference of species found. For Denver Botanic Gardens, an assessment could be created specifically aimed at horticultural species and could format the assessment based on attributes seen in similar problematic species.

There is no question that botanic gardens play pivotal roles in ecological research, community education, and maintaining vast horticultural collections. While Denver Botanic Gardens contains broad horticultural diversity, the potential for non-native species to escape or become invasive cannot be ignored. Creating a watchlist and monitoring species within Denver Botanic Gardens would provide the most benefit to all stakeholders and would allow for future management if necessary.

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