USING SEED SOURCE DIVERSITY AND SPECIES DISTRIBUTION MODELING FOR

EVALUATING GRASSLAND RESTORATION SUCCESS AND SUITABILITY

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Jessica M Lindstrom

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

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SUPERVISORY COMMITTEE:

Jill Hamilton

Chair

Marissa Ahlering

Steven Travers

Jack Norland

Approved:

4/11/2022	Craig Stockwell
Date	Department Chair

ABSTRACT

Seed sourcing for restoration often uses local populations presumed adapted to local environments. This may not be effective under changing climates. Thus, different seed sourcing strategies need testing including multi-source regional collections and their ability to persist in response to change. We compared first-year emergence for single and multi-source seed mixes on plant community diversity following restoration at two locations. There was no discernable effect of seed mix treatment on community establishment. However, land-use history likely has influenced early diversity. Following this, we evaluated which climate variables influence distributions across 26 grassland species' ranges. We produced contemporary species distribution models and evaluated how predicted habitat suitability changed in response to predicted carbon emission scenarios. The climate variables that influenced habitat suitability varied by species with predicted species- and functional-group specific responses to predicted change. These results may aid seed sourcing decisions and identifying regions to implement restorations in the future.

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LIST OF ABBREVIATIONS

MSUM ....Minnesota State University Moorhead

USDA...United States Department of Agriculture

SDSU ....South Dakota State University

MN ....Minnesota

SD ....South Dakota

RSC..Regional Science Center

TNC..The Nature Conservancy

ORD ....Ordway Prairie Preserve

NMDS ....Non-metric multidimensional scaling

PERMANOVA ....Permutational analysis of variance

SDM..Species distribution model

GBIF ....Global Biodiversity Information Database

MaxEnt...Maximum entropy modeling

ESRI..Environmental Systems Research Institute

GCM ....Global circulation model

AUC ....Area under the receiver-operator characteristic curve

CO_2...Carbon dioxide

SSP..Shared socio-economic pathways

CS-Grass ....Cool-season grass

WS-Grass ....Warm-season grass

EB-Forb..Early-blooming forb

LB-Forb..Late-blooming forb

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1. INTRODUCTION

Native grasslands provide essential ecosystem services including hydrological benefits (Seelig & DeKeyser 2006), carbon sequestration (Euliss et al. 2006), nutrient cycling, and habitat for a diversity of species (Helzer & Jelinski 1999; Skagen et al. 2008). Despite the essential ecosystem services grasslands provide, they remain critically imperiled globally (Hoekstra et al. 2005; Comer et al. 2018). One of the largest threats to grasslands has been human-mediated conversion to row-crop agriculture. An estimated 4% and 13% of contemporary remnant Northern Tallgrass Prairie and Northern Mixed-grass Prairies respectively remain today (Comer et al. 2018). Additionally, the rate of conversion is rapidly accelerating to meet the demand of modern agriculture. Conversion rates for Minnesota tallgrass prairie quadrupled during the 2008-2012 time period, and contemporary estimates of remaining grasslands may be much lower than previous estimates (Lark et al. 2019). Anthropogenic conversion has led to increased habitat fragmentation and invasion by exotic species with lasting negative effects to biodiversity and species richness throughout grassland ecosystems (DiAllesandro et al. 2013; Haddad et al. 2015). For example, fragmentation and disturbance often lead to shifts in grassland communities where non-native species may readily establish through competitive advantages in nutrient uptake, creating nutrient-depleted habitats and reducing the native biodiversity (D'Antonio & Mahall 1991; D'Antonio & Meyerson 2002; Eskelinen et al. 2021). To maintain grassland ecosystems and ensure they have the capacity to establish and persist over time, ecological restoration is necessary.

Ecological restoration, defined here as environmental repair of degraded or damaged ecosystems to maintain and mitigate the loss of associated ecosystem services, has been widely implemented and established as a field (Woodworth 2006; Gann et al. 2019). Restoration then

often refers to the activity that practitioners implement to recover ecosystem functions that may have been lost (Gann et al. 2019). A common goal for most restorations is to restore or re-establish plant functional diversity to maintain key biological interactions (Montoya et al. 2012; Barr et al. 2017). Traditionally, ecological restoration has relied on historical information or appropriate reference ecosystems to guide restoration practices necessary to achieve this goal (Miller & Hobbs 2007; Perring et al. 2015; Gann et al. 2019). More recently ecological restoration has incorporated consideration of the abiotic conditions, biodiversity, ecosystem function, and maintenance of evolutionary potential in restoration planning (Montoya et al. 2012; Perring et al. 2015). In an era of rapidly changing climate, these factors are critical as climate change has exacerbated the effects of fragmentation and biodiversity loss (Leimu et al. 2010; Rice & Emery 2003), impacting the resilience of native and restored grasslands (Etterson & Shaw 2001).

Climate change projections for the Northern Great Plains region predict an increase in interannual temperature and precipitation patterns, including extreme climactic events such as heavy precipitation and heat waves that will occur with high degrees of variability (Shafer et al. 2014; Kluck et al. 2018). Predicted increases in winter and spring precipitation could increase runoff and flooding, with extended periods of drought in the summer and fall (Shafer et al. 2014). The variability and unpredictability in timing for these events may potentially alter the timing of important reproductive events for plants, and can increase competition from an abundance of invasive species (Kluck et al. 2018). Experimental work has already shown changes in climate have impacted phenology (Whittington et al. 2015; Dunnell & Travers 2011). Advanced phenology may lead to reduced plant reproductive success if flowering time is mismatched with pollinator availability. Growing seasons may also shift, allowing invasive species

to establish at faster rates. One invasive species of concern, Kentucky bluegrass (*Poa pratensis*), may be able to take advantage of a longer growing season, displacing native flora (DeKeyser et al. 2015). For restorations aiming to restore biodiversity and maintain ecosystem services, management practices will need to be adaptive to ensure plant communities are resilient to change (Perkins et al. 2019). Resiliency may be maintained by a species' ability to cope with changing conditions, adaptation and through phenotypic plastic responses (Funk et al. 2008), resistance to change or invasion (Grime et al. 2008), or dispersal to suitable habitat (Hargreaves et al. 2014).

Under changing environmental conditions, ensuring restorations are resilient and have the capacity to persist and adapt to change is imperative. In this research we focused on two key components of ecological restoration success: i) We empirically evaluated the role of seed source diversity for influencing plant establishment during the initial phases of restoration, and ii) we identified climate factors that influence grassland species presence on the landscape and modeled habitat suitability for those species under current climatic conditions and those predicted under climate change.

1.1. Genetic Diversity in Restoration

Genetic variation is a pre-requisite for adaptive evolution. Therefore, the maintenance of genetic variation within populations is necessary for species to adapt to changing environmental conditions. Genetic variation may be lost through random fluctuations in population size via genetic drift, or maintained through gene flow among populations (Reed & Frankham 2003). In small isolated plant populations that exhibit reduced connectivity or gene flow, decreases in genetic variation or increased relatedness due to interbreeding among related individuals may be observed (Leimu et al. 2010). These isolated populations may also show increased genetic

differentiation which could underlay adaptive differences across a species' distribution (Durka et al. 2017; Bucharova, Michalski, et al. 2017a). If seeds are sourced locally for restoration from small, isolated populations then individual seed sources may not have the requisite genetic variation needed to adapt to change (Davis et al. 2005; Etterson & Shaw 2001). Ensuring the maintenance of evolutionary potential therefore requires seed sourcing strategies that maintain or enhance genetic diversity. Accounting for the role evolutionary forces may play in the maintenance of diversity will aid in the establishment of seed mixes that will ultimately increase restoration success (Bucharova, Michalski, et al. 2017b; Hamilton et al. 2020). Therefore, to ensure short- and long-term success of restorations under changing conditions, the future of seed sourcing for restoration mixes should consider within-species genetic diversity.

Multiple strategies for sourcing seeds to restore biodiverse and functional plant communities have been proposed, and each of these strategies have benefits and drawbacks when considering adaptation to current and future conditions. Current strategies used to establish seed mixes often advocate a 'local is best' approach, or "local provenancing" (Broadhurst et al. 2008; McKay et al. 2005). This approach assumes seeds sourced from the restoration site should outperform seed collected from locations and climates farther from the restoration site (Kawecki & Ebert 2004; Hoban et al. 2016; Bucharova, Durka, et al. 2017). While there is evidence of local adaptation for many plant species (Leimu et al. 2010; Hereford 2009), the degree or scale of adaptation is often unknown, and may be difficult to quantify for species with large ranges (McKay et al. 2005). If seed for restorations is only collected locally and local sites are highly isolated, then they may not contain the necessary genetic variation needed for populations to persist nor the capacity to adapt to new conditions (Davis et al. 2005; Etterson & Shaw 2001). Additionally, considering a rapidly changing climate, locally adapted genotypes may be

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maladapted to the new conditions (Aitken & Whitlock 2013; Hamilton et al. 2015). Therefore, to facilitate adaptation under climate change, alternative strategies such as assisted gene flow or a regional admixture approach are also possible strategies for seed sourcing.

Assisted gene flow is defined as intentional relocation of individuals within their native range to facilitate adaptation to anticipated climate conditions and may be used to supplement genetic variation needed to adapt and maintain evolutionary potential among populations (Aitken & Whitlock 2013). This strategy relies on climate change predictions and aims to maximize the chance seed sourced will be pre-adapted to future climate. However, this strategy is generally considered risky due to potential maladaptation and outbreeding depression (Prober et al. 2015; Aitken & Whitlock 2013). Another strategy, defined as 'regional admixture provenancing' aims to reduce the risk of maladaptation and maximize adaptive capacity by increasing genetic variation through mixing regional seed sources. This strategy mixes seed from multiple regional populations to hedge bets between maintaining local adaptation and assisting gene flow (Bucharova et al. 2019). By increasing the number of populations within a mix this may be used as a proxy for increased genetic variation within individual species used within restoration seed mixes (Jordan et al. 2019). Regional admixture provenancing, therefore, provides a means to ensure local adaptation to the local environment is considered, while also accounting for the genetic variation necessary for evolutionary potential within the restoration.

Despite recommendations for alternate seed sourcing strategies for restoration under climate change, few studies have empirically evaluated their effectiveness in the field. In this study I will test a regional admixture approach using seed collected from two separate regions to create distinct seed mixes of single sources (seeds are collected from one source population), and multiple sources (seeds are collected from multiple source populations). These seed mix types

were used in regional experimental restoration plots to evaluate how seed mix type influenced plant establishment in the first year following restoration.

Replicated experimental restorations can be useful to evaluate and inform restoration techniques for broader application. Experimental restorations provide an ideal means to test restoration strategies in natural environments and to understand how different seeding methods or mixes may affect vegetation structure and composition (Yurkonis et al. 2010), how seed mixes may help to combat invasive species (Norland et al. 2013), or how species composition differs between restorations and remnant prairies (Polley et al. 2005) to influence restoration success. Previous meta-analyses suggest that successful restorations will restore vegetation structure and increase species richness and abundance to preserve ecosystem function (Ruiz-Jaen & Aide 2005; Wortley et al. 2013). My short-term study quantifies restoration success as species diversity following first-year emergence within a restoration. Species diversity can be measured by multiple indices that include estimates of species richness, evenness, and species abundance; all of which are associated with enhancing plant productivity and long-term persistence (Martin et al. 2005; Polley et al. 2003). These estimates may be monitored repeatedly to evaluate how restorations change over time. Using these metrics in our experiment can give us a baseline understanding for how single and multiple seed sources establish during the early stages of restoration. I can then continue to monitor these experimental plots over time to evaluate whether they produce plant communities that are able to adapt to change and remain successful over time.

In this thesis, I will test the regional admixture approach to seed sourcing by evaluating the impact single and multi-source seed mixes have on early establishment within a restored grassland context. I predict that multiple-source seed mixes will have increased species establishment and diversity compared to single-source mixes.

1.2. Species Distribution Modeling to Inform Restoration Decisions

Species distribution models (SDMs) are a commonly used mapping tool that is widely used in habitat, wildlife and resource management, restoration and population ecology, and conservation planning (reviewed in Franklin 2013; Elith et al. 2011). SDMs can be used to understand the relationship between abiotic climate factors that underlay a species' ecological niche and can be used to predict the probability of species presence across a landscape in contemporary and future climate conditions (Elith & Leathwick 2009; Elith et al. 2011). The natural distributions of species are limited by several factors: 1) the biotic conditions 2) the abiotic conditions and 3) the movement or dispersal capacity of a species (Hutchinson 1957; Soberon & Peterson 2005). The abiotic and biotic conditions needed for a species to persist can create an idealized version of a species' "fundamental niche", the entire area a species might occupy. As described by Hutchinson (1957), however, the fundamental niche is often less restrictive and realistic than a species' "realized niche". The realized niche of a species considers external forces, such as competitive interactions or barriers to dispersal, which may limit the potential distribution of a species (Soberón & Nakamura 2009). Ecological niche modeling often takes into consideration these three sets of factors to broadly conceptualize differences in where a species could exist (fundamental niche) versus where a species actually exists (realized niche) to make considerations about species ranges across timescales (Peterson 2006). Although all three factors are important to understanding a species' range, incorporating biotic and dispersal information often requires large amounts of observational data that may be rare to find and its efficacy within modeling is still uncertain for modeling large-scale change (Lasky et al. 2020; Detto et al. 2019). Therefore, modeling that describes the "climatic niche" is one of the most

common ways to realistically represent a species' niche within geographic space (Araújo &

Luoto 2007; Peterson & Soberón 2012; Melo-Merino et al. 2020).

The core assumption with climatic niche modeling assumes that local climate is one of

the best descriptors for explaining species geographic distribution and constraints on those

ranges, and indeed there is evidence that species' ranges are supported and constricted by local

environmental climatic conditions (Sexton et al. 2009; Pearson & Dawson 2003). To understand

which climate factors drive species persistence in an area, models correlate where a species

currently exists, the realized niche, with the climate at that environment (Pearson & Dawson

2003). Under perfect conditions a realized niche would be considered at equilibrium with

climate, or other abiotic conditions, when a species is able to occupy all the environmental areas

where climate is suitable and is absent everywhere that climate is not suitable (Araújo & Pearson

2005). Although true equilibrium is rarely met, previous studies have found that plant species

distributions can be accurately predicted using solely climate data describing the past, present,

and future climatic niches (Araújo & Pearson 2005; Pearson & Dawson 2003). This may be a

result of plant dispersal capabilities being adequate to allow plants to occupy the suite of habitats

that are suitable (Araújo & Pearson 2005); however, it is important to note that these models

exclude other factors that potentially alter how species establish across landscapes (Araújo &

Peterson 2012). SDMs using climate as the main predictors influencing species distributions and

have been used to inform conservation decisions, including predicting habitat suitability and

producing seed transfer guidelines, which may be necessary as climate change alters the

relationship between climate and species' success (Gray & Hamann 2011; Potter & Hargrove

2012). If we assume that local climatic variables structure the distribution of species on the

landscape, then we can use climatic information of known species occurrences to understand

those climatic variables that predict where a species is currently observed and where it may persist in the future. Understanding species-climate relationships may be crucial to consider within restoration projects to ensure these relationships are maintained for species to persist under changing conditions. Additionally, as the number of restoration projects increase so does the need for diverse and adaptable plant seed to complete these restoration projects. To meet these demands SDMs can identify where species may exist currently as well as in the future to target seed sourcing efforts from highly suitable areas (Havens et al. 2015; Potter & Hargrove 2012).

In this study, we use the geographic presence data of 26 common grassland species and the associated climate at those points to identify the components of precipitation and temperature, that influence each species' distributions across its entire range. We predict that individual species will be influenced by similar components of climate, but not all variables will contribute similarly across species. We then used these relationships between species presences and associated climate to create estimates of habitat suitability across the predicted ranges for contemporary climate. These maps were then used to quantify how suitability will change across two forecasted scenarios of climate change for high and low carbon emissions. We predict that across species and functional groups that habitat suitability change will be variable due to the independent and unique relationships each species exhibits with local climate. These results may influence where restoration practitioners collect seed for restoration projects to maximize the likelihood of long-term species persistence and restoration success and may identify areas where habitat maintains high species suitability across climate change scenarios.

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2. SEED SOURCING FOR CLIMATE RESILIENT GRASSLANDS: THE ROLE OF SEED SOURCE DIVERSITY DURING EARLY RESTORATION ESTABLISHMENT

2.1. Abstract

Restoration often advocates for the use of local seed in restoration; however, increasingly new strategies have been proposed to incorporate diverse sources to maintain evolutionary potential within seed mixes. Increasing seed sources per species within a seed mix should increase genetic variation, however, few empirical studies have evaluated how seed source diversity impacts plant community composition following restoration. Thus, the goal of this research was to compare the use of single or multi-source seed mix treatments to plant community diversity following restoration. Using 14 species commonly applied in grassland restoration, I examined plant community diversity following restoration comparing seed mixes with either one or five sources per species across two restoration sites in Minnesota and South Dakota, United States. Following seeding, species establishment and abundance were recorded to calculate plant diversity for each seed mix treatment. There were no major effects of seed mix treatment on community emergence and diversity observed, with the majority of plant establishment reflecting non-seeded species. However, site-specific differences were observed. Heterogeneous land-use history associated with the Minnesota site likely contributed to differences across the restoration treatments. In contrast, community diversity at the South Dakota site was homogeneous across seed mix treatments with changes in plant community influenced solely by early season species establishment. This suggests land-use history irrespective of seed mix treatment influenced establishment and persistence, particularly in the first year following restoration. Future monitoring across seasons will be needed to evaluate if community diversity changes in response to seed mix treatment.

2.2. Introduction

One of the major aims of ecological restoration is to restore or re-establish functional plant species diversity to ensure key ecosystem services are maintained (Barr et al. 2017; Montoya et al. 2012). To ensure ecosystem health and the maintenance of productive plant communities, this includes creating diverse seed mixes for application in restoration (Tilman et al. 1996, 1997, 2001; Brudvig 2011). These seed mixes create communities that may be resilient to changes in nutrient availability (Craven et al. 2016), competition from non-natives (Funk et al. 2008; Oakley & Knox 2013; Yurkonis et al. 2012; Norland et al. 2013), and climate change (Isbell et al. 2015). Evolutionary theory emphasizes the important role both inter- and intraspecific variation established within seed mixes may have to restoration success over time (McKay et al. 2005). Greater biodiversity within restoration communities may increase total plant productivity across time leading to increased stability in soil nutrient availability (Craven et al. 2016), and resilience to extreme events (Isbell et al. 2015). In addition, intraspecific variation is essential as this may provide the raw material that natural selection may act upon and is needed to maintain species' evolutionary potential (McKay et al. 2005; Zeldin et al. 2020). Despite the importance of intraspecific diversity to restoration success, few studies have quantified the role diversity within species has to restoration outcomes (Hamilton et al. 2020). Consequently, to ensure that plant communities persist over time and in response to change, there is a need to consider both the role of within and between species diversity to restoration.

Current strategies used to establish seed mixes often advocate a 'local is best' approach (Broadhurst et al. 2008; McKay et al. 2005). This approach assumes that local seed sources will have greatest fitness in local restoration environments relative to non-local sources (Kawecki & Ebert 2004; Hoban et al. 2016). While there is evidence of local adaptation for many plant

species (Leimu et al. 2010; Hereford 2009), the degree or scale of adaptation is often unknown (McKay et al. 2005). Furthermore, to conserve evolutionary potential requires genetic variation (Kawecki & Ebert 2004). Genetic diversity is the raw material that selection acts upon and is necessary for adaptation to changing environmental conditions. Genetic variation may be lost through random fluctuations in population size via genetic drift, but maintained through gene flow among populations (Reed & Frankham 2003). In addition, small, isolated plant populations that exhibit reduced connectivity or gene flow may exhibit reduced genetic variation, but increased genetic differentiation (Durka et al. 2017). If seeds are sourced locally for restoration from small, isolated populations then individual seed sources may not have the requisite genetic variation needed to adapt to change (Davis et al. 2005; Etterson & Shaw 2001). To ensure the maintenance of evolutionary potential therefore may require seed sourcing strategies that increase genetic diversity. Accounting for the role evolutionary forces play in the maintenance of diversity will aid in establishing seed mixes that ultimately increase restoration success (Bucharova et al. 2017; Hamilton et al. 2020).

To ensure preservation of evolutionary potential, variation within species is required alongside the establishment of species rich seed mixes. The combination of intraspecific and interspecific species diversity can influence community composition during establishment (Larson et al. 2013). Diversity at these two scales can impact short-term response to the environment and competition with local seed banks (Grman et al. 2013). During the first few years following restoration it is expected that communities will be largely dominated by non-seeded weedy species typically found within the soil seed bank (Bakker et al. 1996). For example, when comparing an ongoing prairie restoration to multiple remnant prairies, Martin et al. (2005) observed more non-native species present within the restoration, with the overall

proportion of non-natives ranging from 236% to 413% higher in the restoration relative to remnant sites. Thus, monitoring early establishment of seeded relative to non-seeded species may be important to predicting how long-term plant communities may change compositionally . Despite the potential importance of early establishment to long-term restoration success, this phase is often overlooked in favor of evaluating restorations after they have been established for several years.

Globally, native grasslands remain one of the most critically imperiled ecosystems requiring active restoration (Hoekstra et al., 2005). These ecosystems provide essential services, including maintenance of hydrological flow and retention (Seeling & DeKeyser 2006), carbon sequestration (Euliss et al. 2006), nutrient cycling, and habitat for a diversity of species (Helzer & Jelinski 1999; Skagen et al. 2008). Throughout the North American Great Plains, up to 87% of historical grassland habitat has been lost primarily to agricultural conversion (Comer et al. 2018; Hoekstra et al. 2005; Samson et al. 1999) leading to highly fragmented and isolated remnant habitats. Where these grasslands remain, they are prone to invasion by non-native species and the evolutionary consequences of isolation, which has lasting negative effects to diversity and species richness (DiAllesandro et al. 2013; Haddad et al. 2015). Ensuring seed mixes restore grassland populations so they have the capacity to adapt to change, resist invasion, and persist over time is critical. However, the role of intraspecific diversity within seed mixes to restoration success has yet to be empirically evaluated. Therefore, it is necessary to consider the impact of both species and population diversity within seed mixes has to establishment of grassland restorations.

We assessed plant community diversity following restoration using single- and multi-source seed mixes to test the role within-species seed source diversity played in community

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establishment. We used seed collected from five unique populations for each of 14 different species as a proxy for creating genetic diversity within a seed mix. We expected that increasing the number of unique seed sources per species used within a seed mix would lead to increased emergence diversity following restoration relative to the use of a single seed source seed mix (Bucharova et al. 2019). Overall, we predicted greater within-species diversity for seed mixes would lead to increased species diversity in restored plant communities. This research empirically evaluates the role of within species to between species diversity following restoration. This study will provide a baseline understanding of the role of diversity across scales to establishment during restoration.

2.3. Methods

2.3.1. Seed Collection

In the summer of 2019, seed from 12 forb and two grass species were collected between June and October from remnant native prairies within the Northern Great Plains of the United States. A minimum of five unique populations per species each were collected from the Missouri Coteau region of North and South Dakota and from the northwestern prairie region of Minnesota (Table 2.1, Figure 2.1). These 14 species were chosen because they are widely distributed throughout the Northern Great Plains and are commonly used in regional restoration seed mixes (e.g., Smith 2010; Kurtz 2013). In addition, to control for potential dominance of warm-season grasses and to increase establishment of sown forbs, species chosen were weighted toward forb species (McCain et al. 2010; Norland et al. 2013; Dickson & Busby 2009). Populations were classified as distinct if separated by at least one mile, however, were more commonly spaced further apart. In northwestern MN, distances between seed source locations ranged from 3 km to 215 km (Table A2), and pairwise distances between the restoration site and seed source ranged

from 2 km to 129 km (Table A3). Within the Missouri Coteau region, distances between seed source locations ranged between 2 km and 312 km (Table A4), and pairwise distances between the restoration site and seed source site ranged from 3.5 km to 214 km (Table A5).

Seed was hand-harvested as it ripened, with seed harvested multiple times at different sites throughout the growing season following Bureau of Land Management seed harvesting guidelines (BLM 2015). Within each population, individual maternal seed heads were sampled at least three feet apart to reduce potential relatedness within populations. For species with multiple seed heads, no more than 30% of available seed per maternal seed head was collected.
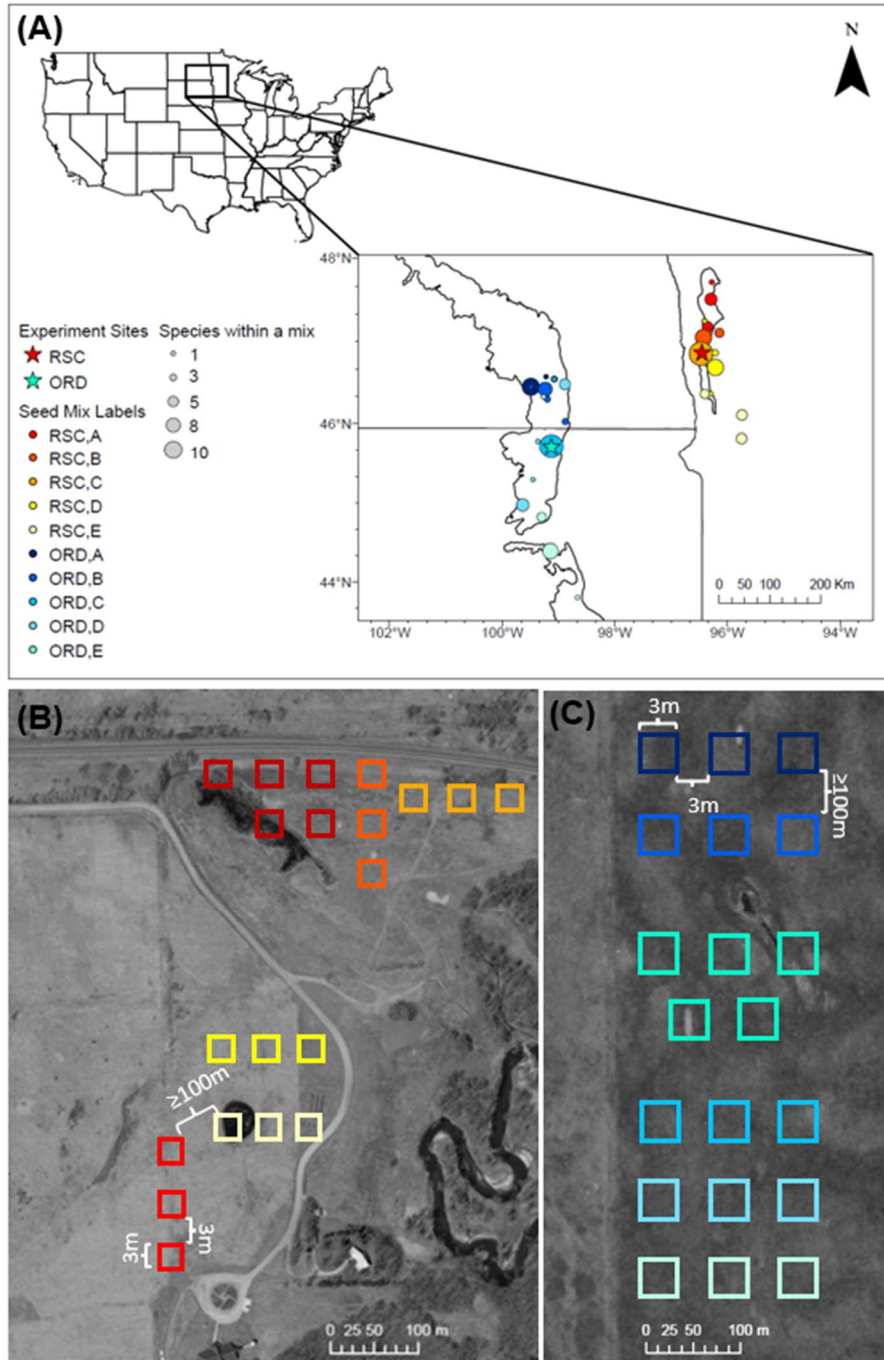
Figure 2.1. A) Seed collection sites for seed mix treatments for Missouri Coteau (blues) and northwestern MN (reds) regions respectively. Colors represent individual seed mixes, and proportional symbols indicate the number of species sourced from a single site that was used within a seed mix. Stars indicate experimental site locations. B) Experimental plots layout at RSC in Glyndon, MN. C) ORD experimental plots layout at ORD in Leola, SD. Colors correspond to seed treatment, single source treatments include three replicate plots and the five-source treatment includes five replicate plots.

Table 2.1. Species used in experimental restoration plots for RSC and ORD sites, weighed amounts used in individual seed mix treatments, individual species composition within seed mixes, approximate seeds/m^2, and seed viability included where applicable.

Species Scientific Name	RSC					ORD			
	Single-population Seed Mix (g)	Five-population Seed Mix (g)	Species composition in mix (%)	Seeds/m^2	Viable Seed (%)	Single-population Seed Mix (g)	Five-population Seed Mix (g)	Species composition in mix (%)	Seeds/m^2
Amorpha canescens	12.5	2.5	21.8	784	20	12.5	2.5	23.2	784
Anemone cylindrica	7.5	1.5	13.1	764	82	-	-	-	-
Artemisia frigida	0.5	0.1	0.9	556	62	-	-	-	-
Bouteloua curtipendula	5.0	1.0	8.7	233	-	1.5	0.3	2.8	70
Bouteloua gracilis	-	-	-	-	-	0.5	0.1	0.9	78
Dalea purpurea	5.0	1.0	8.7	353	-	5.0	1.0	9.3	353
Echinacea angustifolia	8.0	1.6	13.9	219	-	8.0	1.6	14.8	219
Geum triflorum	1.3	0.3	2.2	132	47	8.5	1.7	15.8	899
Helianthus maximiliani	1.3	0.3	2.2	64	-	1.0	0.2	1.9	51
Helianthus pauciflorus	2.0	0.4	3.5	31	-	2.0	0.4	3.7	31
Hesperostipa comata	-	-	1.5	-	-	0.6	0.1	1.2	18
Liatris punctata	4.3	0.9	7.4	117	-	1.0	0.2	6.5	274
Pediomelum argophyllum	2.5	0.5	4.4	88	-	3.5	0.7	1.9	27
Potentilla arguta	1.5	0.3	-	1352	88	1.3	0.3	6.5	123
Ratibida columnifera	-	-	2.6	-	-	2.5	0.5	2.3	1127
Schizachyrium scoparium	2.8	0.6	4.8	162	-	-	-	4.6	412
Solidago rigida	2.5	0.5	4.4	402	54	2.5	0.5	4.6	402

2.3.2. Seed Mix Preparation

Following harvest, seeds were dried at room temperature for a minimum of two weeks and then transferred to 4□ storage for seven months to provide cold stratification and maintain viability. Seeds were cleaned using several species-specific approaches. Large seeds were stripped by hand, smaller seeds separated using sieves, *Hesperostipa comata* (Needle and thread grass) seed awns were trimmed during the drying process to limit tangling, and *Solidago rigida* (Stiff Goldenrod) and *Helianthus maximiliani* (Maximilian sunflower) and *H. pauciflorus* (Stiff sunflower) seed were mechanically cleaned and separated using a Fractioning Aspirator Test Model at the USDA Agricultural Research Center in Fargo, ND.

Seed was weighed for each species (Mettler Toledo, ML503T/00) from each population to calculate population-specific numbers of seeds using a seeds per gram conversion (Table A1). To maximize the seeds per species in the mix and ensure seed mix consistency across treatments and replicates, the amount of seeds to include in the mix per species was calculated based on the population with the lowest seed weight (g). In addition, for *Artemisia frigida* (Fringed sagewort), *H. pauciflorus*, and *S. rigida*, the amount of seeds used in the seed mixes was reduced by 0.9%, 3.5-7.0%, and 4.4-6.0% of the lowest seed weight respectively, to ensure these species were not overrepresented in seed mixes as they can exhibit dominant characteristics (Table 2.1).

Across the two regions, seed mixes were established using the same species with the exceptions of *A. frigida*, *Anemone cylindrica* (Tall timbleweed), and *Schizachyrium scoparium* (Little bluestem), which were collected and planted exclusively in the northwestern MN region and *Ratibida columnifera* (Prairie coneflower), *H. comata*, *Bouteloua gracilis* (Blue grama), which were collected and planted exclusively in the Missouri Coteau region. Five different seed mixes were established, each using a single unique population per species for the seed mix

within each of the two restoration regions. For the single-source seed mixes, populations for the different species were largely sourced from similar latitudes to minimize potential impacts associated with latitudinal variation in phenology (Olsson & Ågren 2002; Dunnell & Travers 2011) (Figure 2.1). In addition to five single source seed mixes, one multiple-source seed mix was established for each region. The multi-source seed mix consisted of proportionally the same amount of seeds per species as the single-source mix, but each species' contribution was divided evenly across five population sources. Thus, for both single and multi-source seed mixes the proportion of seed used per species was the same. In this way, the ratio of species present within the single source and multi-source was maintained across seed mixes for direct comparison. Vermiculite (Vigoro) was added to final seed mixes in a 1:1 ratio as a common method to increase seed to soil contact during planting and thus increase probability of emergence (Shaw et al. 2020).

2.3.3. Seed Viability

Unused seed from the restoration plots sampled from the northwestern MN region were sent to South Dakota State University's Seed Testing Laboratory to assess seed viability. Unused seed from the Missouri Coteau were not available for seed viability testing. These tests evaluated the total viability of individual species when grown under ideal laboratory growth conditions to induce germination. This test reported the percent of seed that germinated defined as the total number of individuals emerged per seeds planted, percent of hard seed defined as seed that is dormant due to a water impervious seedcoat, and dormant seed which is defined as seed that is viable but does not germinate due to a physical or physiological condition (SDSU Seed Testing Laboratory; https://www.sdstate.edu/sites/default/files/file-archive/2021-07/Seed-Testing-Lab.pdf).

2.3.4. Restoration Sites and Site Preparation

During May and June of 2019, experimental restoration sites were identified and prepped in both the northwestern MN and Missouri Coteau regions. The northwestern MN restoration site was established at the Minnesota State University Moorhead Regional Science Center (RSC) (46.872, -96.452) in Glyndon, MN. Portions of this site are abandoned agricultural brome fields that are adjacent to remnant mesic prairie owned by Buffalo River State Park. Another portion of this site was actively maintained as the Ponderosa golf course starting in 1962 and continued operation after the transfer of ownership until May 2015, following which limited mowing management has occurred. Due to site and space limitations, both areas of this site were used to establish the experimental plots. The Missouri Coteau restoration site was established on the Samuel H. Ordway Prairie Preserve (ORD) (45.704 -99.086), owned and managed by The Nature Conservancy (TNC). Prior to TNC ownership in 1978, this site was used as a brome/alfalfa production plot for cattle. Since TNC's ownership, this site has been maintained for hay production every other year.

In 2019, the RSC site was prepared by placing landscape cloth over experimental restoration plots to remove existing vegetation and limit potential establishment and competition with the existing seedbed prior to applying the restoration treatment. In fall 2019, the ORD site was treated with herbicide prior to application of restoration treatment (Roundup®, 3-4% concentration) within each plot to reduce competition with existing weedy vegetation during establishment. Additionally, all plots had a second Roundup treatment in early May, 2020 to further reduce *Bromus inermis* (Smooth brome) encroachment.

At each site, twenty 3 x 3m experimental restoration plots were established. This included establishment of five different single source seed treatment plots each replicated three

times (n=15) and one multi-seed source treatment replicated five times (n=5). For each individual replicated plot within a seed treatment, a barrier of 3m was maintained and a minimum 100m buffer maintained between each single- and multi-source seed treatment group to limit potential gene flow between plots.

2.3.5. Planting Experimental Restoration Treatments

To establish the restoration treatments, tarps were removed from the plots at the RSC site, and litter was raked and hand weeded in April 2020 at both sites to expose the seed bed. Following this, each plot was broadcast seeded and then raked again to increase seed-soil contact. For both sites, five times the total commonly recommended seeding rate of ~5kg (11 pounds) of seeds per acre were applied to increase probability of emergence success (Rowe 2010). Higher seeding rates were applied as these rates have previously been associated with increased establishment and diversity following restoration (Sheley & Half 2006; Barr et al. 2017). An agri-fab push lawn roller was used to increase seed to soil contact and enhance the probability of germination success. To limit potential carryover of seeds between seed treatments the roller was rinsed and dried between each application. Finally, each plot received a one-time watering treatment. Throughout the growing season, plot maintenance included weekly barrier mowing around each plot. In July, mid-season mowing was performed at both sites to increase light availability and reduce competition with non-seeded species (Maron & Jefferies 2001; Kaul & Wilsey 2020). Plots were mowed at the maximum adjustable height setting (12.7cm) and all trimmings were removed.

2.3.6. Data Collection

Each restoration plot was visited once per month at both sites between June and September of 2020 to assess plant community composition. A 0.2m x 0.2m quadrat randomly

placed at each of the four cardinal corners and center of each replicated experimental plot was used to estimate community composition of the broader restoration plot. To reduce the impact of edge effects, quadrats were not placed directly at the edges of each plot. For all species present in the quadrat, we counted the number of individuals present and estimated the percent cover per species. Individuals that were unidentifiable in the field were marked with unique toothpicks and photographed for later identification. There were two unknown species at the RSC site and three at the ORD site that did not match planted species seedlings and were unable to be identified. These species were uniquely labeled as unknowns and included in diversity calculations as unique non-seeded species. Total percent cover of dead vegetation and percent bare soil cover was also assessed visually within the quadrat. At the quadrat-level, total species coverage was recorded as the total percent coverage of each species, litter coverage was the percent cover of dead matter covering the ground, and soil coverage was the percent of visible bare ground. Each coverage estimate was assessed with a modified Daubenmire cover-class system for grassland vegetation (Table A6; Daubenmire 1959) and averaged across quadrats to obtain replicate-level percent coverages for each seed mix treatment.

2.3.7. Statistical Analysis

To infer plant productivity and assess plant community composition following restoration, species diversity metrics such as richness, evenness, abundance, and associated diversity indices are often used and may be monitored over time (Martin et al. 2005; Polley et al. 2003). We tested for differences in community composition based on seed mix treatment at each of our restoration sites using measures of species richness and diversity. Species richness was defined as the total number of species present across all five quadrats sampled per replicate and abundance as the total number of individuals present per species across quadrats. We also

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analyzed the total number of unique species and the number of seeded species that established within seed treatments for replicated plots. To evaluate our seed treatment communities regardless of planted or non-seeded species status, we calculated Shannon's Diversity Index (H') for each seed treatment and each replicate plot across time from June to September.

$$H' = \sum_{i=1}^{s} p_i \, ln p_i$$

Where s is the total number of species within the community (richness), pi is the proportion of each species (i) within the community relative to the total number of species multiplied by the natural logarithm and summed across all species to get a value between 0-1. Values closer to 0 indicated lower diversity and values closer to 1 indicated higher diversity. We used Shannon's Diversity for our data as it was the most appropriate given our data collection approach (Magurran 2004). Diversity indices were calculated at the seed treatment level and for the individual replicates within seed treatment to create distance matrices.

To compare plant community diversity within each restoration site for varying seed treatments across time we used non-metric multidimensional scaling (NMDS) in three dimensions with a Bray—Curtis dissimilarity distance matrix which was derived from the Shannon's diversity indices for each seed treatment and each month of data collection. We used NMDS because it uses an ordination approach where community data is summarized on two-axes and communities that are more similar cluster together (Ruiz-Jaen & Aide 2005).

To evaluate differences between community compositions, we performed permutational ANOVAs (PERMANOVAS) on the same Shannon's diversity values for seed treatment communities across each month, using the adonis function in package "vegan" (Oksanen et al. 2020). We used a PERMANOVA approach to evaluate differences between individual seed treatments and more broadly between single source and five-source community diversity. Seed

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treatment, replicate, month, and the interaction of seed treatment and month were predictor variables and the percent bare ground and thatch were included as random-effect variables within our models. Post-hoc pairwise comparisons were performed to evaluate differences between seed mix treatment per month for RSC communities and by month for ORD communities within the pairwise.adonis function in package "pairwiseAdonis" (Martinez Arbizu 2019). All analyses were conducted in R version 4.0.2 (R Core Team 2016).

2.4. Results

2.4.1. Seed Viability

Six of the 14 species sent for testing had enough seed for an assessment of viability. Variability in seed viability may impact how individual species may or may not establish within the first year following restoration. Seeds from *Amorpha canescens* exhibited a viability score of 20% with 16% of seed reaching germination, 4% labeled as hard seed, and 0% dormant seed. Seeds from *Anemone cylindrica* exhibited 82% viability with 75% of seed reaching germination, 0% hard seed and 7% assessed as dormant. Seeds from *Artemisia frigida* were 62% viable, with 25% of seed reaching germination, 0% labeled as hard seed, and 37% dormant seed. *Geum triflorum* seed had a total viability of 47% with 47% of seed reaching germination, 0% labeled as hard seed, and 0% dormant seed. *Potentilla arguta* seed exhibited 88% viability, with 66% of seed reaching germination and 0% labeled as hard seed, 22% dormant seed. Finally, *Solidago rigida* seeds had a viability score of 54% with 44% of seed reaching germination, 10% labeled as hard seed, and 10% dormant seed.

2.4.2. Plant Community Structure Following Restoration

Seed mix application at both the RSC and ORD sites resulted in a mixture of seeded and non-seeded species emergence. At the RSC site, seeded species emerged from all plots excluding

seed treatment 'D' in the first growing season. Of seed mix treatment types, the multi-source seed mix type 'ABCDE' had the greatest number of seeded species emerge, including *Echinacea angustifolia*, *Helianthus maximilani*, and *Verbena hastata*. Across all seed treatments at the RSC site, *Helianthus maximilani* exhibited the greatest rate of emergence, followed by *Liatris punctata*. In the first year of observation, only five of the seeded species established at the RSC site. At the ORD site, seeded species emerged within all plots in the first growing season. Of seed mix treatment types, the multiple-source seed mix type 'ABCDE' and the single-source seed treatment 'C' had the greatest number of seeded species emerge, including *H. maximiliani*, *S.rigida* which were found within every seed treatment, followed by *Ratibida columnifera*, and *Dalea purpurea*. In total only six unique seeded species established at the ORD site.

At both restoration sites, seed treatment plots were largely dominated by non-seeded species (Figure 2.2) At the RSC site the most common species within our experimental restoration plots were *Ambrosia psilostachya* (Western Ragweed), *Melilotus* sp. (Sweetclover sp.), *Panicum capillare* (Witchgrass), *Poa pratensis* (Kentucky Bluegrass), *Oxalis stricta* (Yellow Wood Sorrel), *Trifolium repens* (White Clover). At the ORD site the most common species within our experimental restoration plots were *A. absinthium*, *Bromus inermis* (Smooth Brome), and *P. pratensis*.

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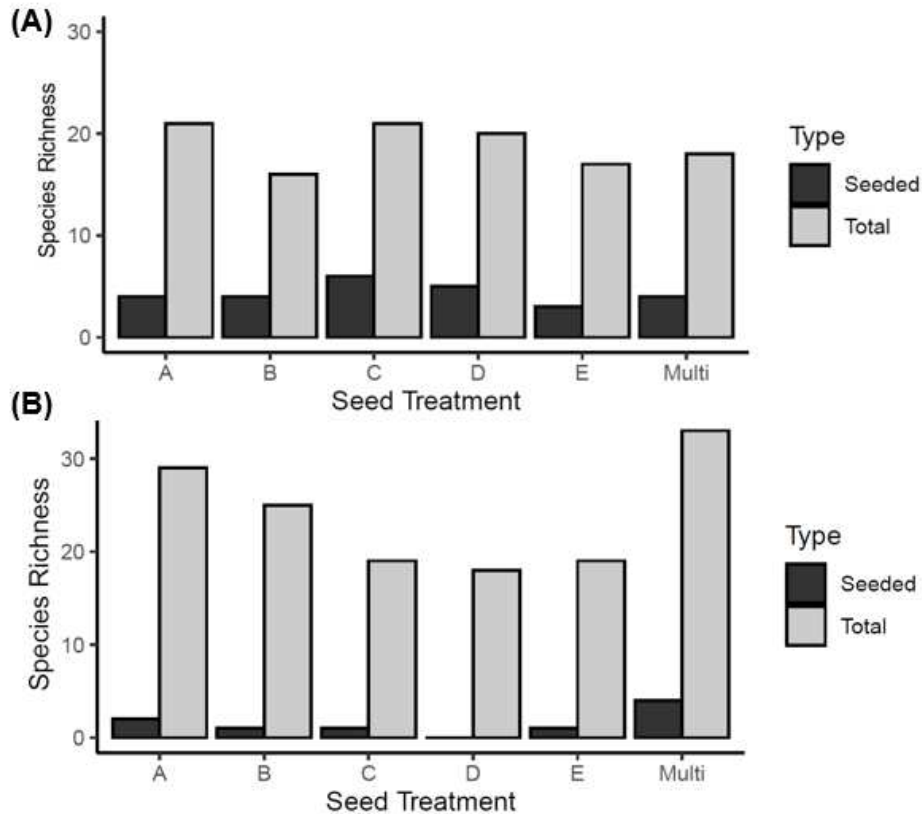
Figure 2.2. Comparison of seeded and total species richness within each seed treatment type throughout June-September 2020 for ORD experimental plots (A) and RSC experimental plots (B). Overall seeded richness was greater within all ORD plots compared to RSC. Total species richness was higher in RSC than in ORD, and the multiple source seed treatment had greatest seeded species richness compared to single source seed treatments.

To evaluate plant community-level differences between seed mix treatment types and across the growing season, we used a PERMANOVA based on Shannon's Diversity. Additionally, to visualize any differences in these plant communities we used an NMDS with Bray–Curtis dissimilarity. Within the RSC site, we found significant community-level differences based on seed mix treatments (pseudo-F= 18.268, p = 0.001;), plot replicate (pseudo-F =7.868, p = 0.001), month (pseudo-F= 2.677, p = 0.018), and the interaction of seed treatment and month (Pseudo-F= 2.172, p = 0.008; Table 2.2). However, as very few seeded species established across seed mix treatments, the differences observed appear to be largely driven by site-level differences associated with spatial heterogeneity in the presence of non-seeded species

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(Figure 2.3B). To then evaluate which seed treatments, or location of seed treatments within the RSC site were compositionally different, we then performed individual pairwise analyses. Pairwise comparisons evaluating community compositions differences across seed mix treatments were subset by month to account for the significant interaction of seed treatment and month found within our PERMANOVA results. From these comparisons we found the five-source seed treatment was significantly different from all single-source seed treatments across all months with the sole exception of seed source 'E, which became more similar to the five-source treatment over time (Table A7). This follows our expectation that the multiple-source treatment would produce a more diverse community when compared to single-source treatments; however, with the caveat that differences observed seem to be driven largely by the diversity of non-seeded species present within individual plots.

Within the ORD experimental restoration site, we found no significant community-level differences between seed mix treatments. However, within our PERMANOVA of community composition based on Shannon's Diversity Index, we observed a significant difference among our ORD communities based on month alone (pseudo-F= 0.385, p<0.001; Table 2.1). These results indicate that any differences in community diversity was not due to seed mix treatments but were primarily explained by the growing season (Figure 2.3A). Pairwise comparisons found that plant community composition in June was significantly different from the later seasonal communities in August and September (Table A8). These results suggest that community diversity observed across the restoration site was different in June than was observed later in the season.
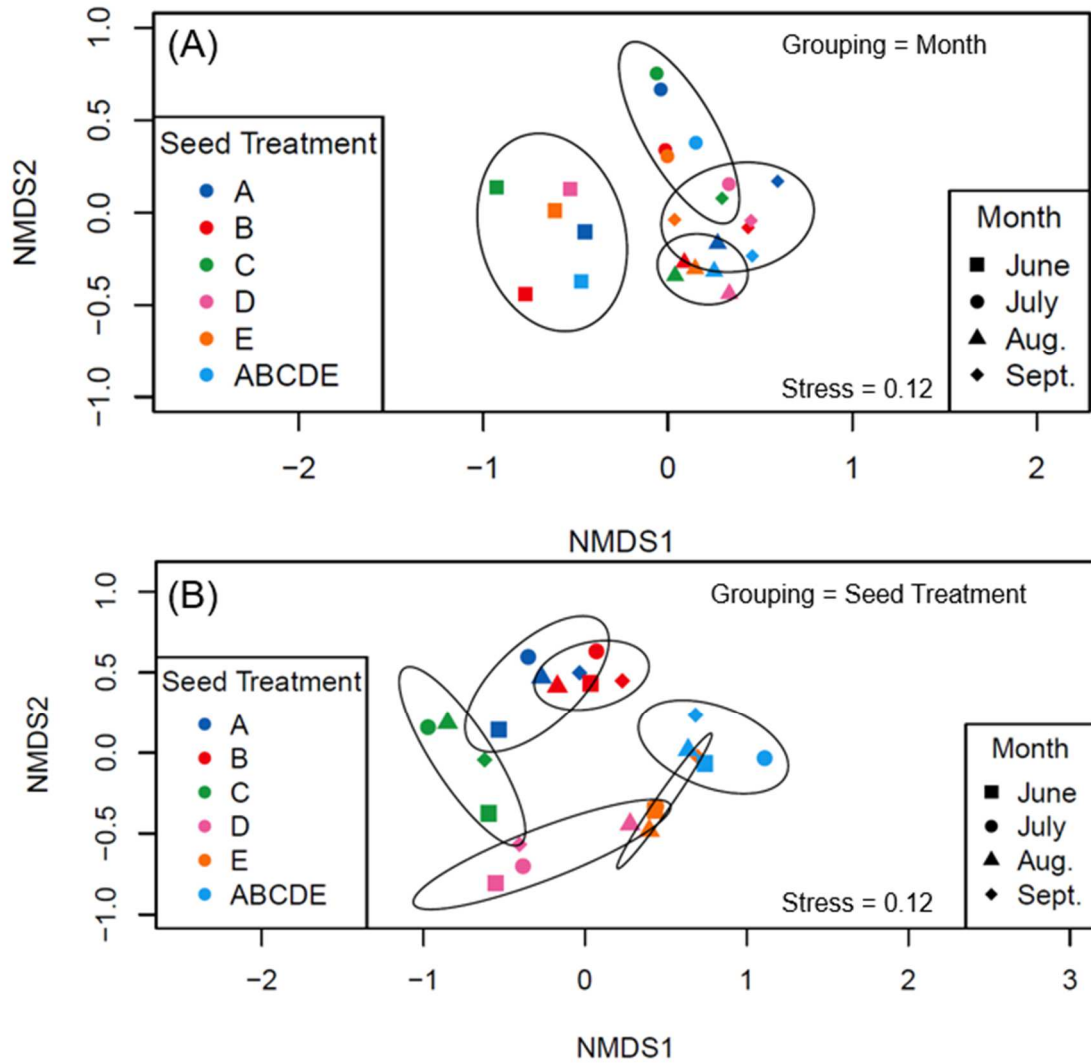
Figure 2.3. Nonmetric Multidimensional Scaling with Bray – Curtis dissimilarity graphs of the first year established communities within (A) ORD plots grouped by month and (B) RSC plots grouped by seed treatment. Seed treatment indicated by color and shapes indicate month of data collection. Ellipses are 95% confidence intervals.

Table 2.2. PERMANOVA results for community composition differences within RSC experimental plots, using Seed Treatment, Plot Replicate, Month, and the interaction between seed treatment and month as main explanatory variables.

	Df	SS	MS	Pseudo F	R2	*P*
Seed Treatment	5	1.475	0.295	18.268	0.323	*0.001*
Plot Replicate	14	1.778	0.127	7.868	0.390	*0.001*
Month	3	0.130	0.043	2.677	0.028	*0.018*
Bare Ground	1	0.002	0.002	0.135	0.000	0.897
Thatch	1	0.007	0.007	0.408	0.001	0.640
Treatment:Month	15	0.526	0.035	2.172	0.115	*0.008*
Residuals	40	0.646	0.016		0.142	
Total	79	4.5627			1	

Table 2.3. PERMANOVA results for community composition differences within ORD experimental plots, using Seed Treatment, Plot Replicate, Month, and the interaction between seed treatment and month as main explanatory variables.

	Df	SS	MS	Pseudo F	R2	*P*
Seed Treatment	5	0.181	0.036	2.165	0.077	0.068
Plot Replicate	14	0.228	0.016	0.975	0.097	0.509
Month	3	0.908	0.303	18.095	0.385	*0.001*
Bare Ground	1	0.018	0.018	1.079	0.008	0.314
Thatch	1	0.001	0.001	0.061	0.000	0.928
Treatment:Month	15	0.352	0.023	1.401	0.149	0.157
Residuals	40	0.669	0.017	0.284		
Total	79	2.358	1.000			

2.5. Discussion

Current local seed sourcing approaches during restoration may not adequately incorporate within species genetic diversity needed to re-establish functional plant communities for adaptation to changing environmental conditions. Thus, establishing diversity within and between species for seed mixes will be critical to ensuring restoration success. Using seed source as a proxy to indicate increased genetic variation, we have empirically evaluated how community diversity establishes following the use of single and multiple- source seed mix treatments. There was no major effect of seed mix treatment type on increasing community diversity within the

first year following restoration at two sites. However, community diversity across seed mix treatment types at this early stage following restoration was strongly influenced by spatial heterogeneity and by the growing season across the RSC restoration site, and strongly influenced by time at the ORD site. Community diversity within both sites was largely dominated by non-seeded species, with limited emergence of seeded species within the first year. These observations are consistent with previous restoration studies, which observed that non-seeded species may dominate restored environments during the first several years following restoration before seed mix species are able to establish (Kaul & Wilsey 2020). Although no differences were observed in community diversity between our single and multiple-source seed mix treatments, our results suggest that first-year restoration communities are influenced by heterogeneity in a restoration site and temporally by the growing season. Thus, land-use history may be important in influencing plant establishment and persistence over time, particularly in the first year following restoration.

2.5.1. Seed Viability

Although non-seeded species were expected in the first year, variation in seed viability within our seed mixes (ranging from 20-88% for the RSC site) may have impacted first year emergence. For seed viability testing, 7-37% of seeds were considered "dormant" and therefore may have germinated within the first year but could emerge in subsequent years provided that environmental conditions in the future are favorable for germination. In addition, seed predation and seedling herbivory may have reduced establishment success during the first year. Herbivore disturbance can mediate non-seeded species dispersal through selective seed herbivory on native plant species (Howe & Brown 2000) which may affect overall species diversity. At the northwestern MN site, the thirteen lined-ground squirrel (*Ictidomys tridecemlineatus*) was

observed, alongside nearby and within-plot gopher mounds. As our study design was aimed to mimic natural restoration practices, we did not take measures to actively exclude mammals from the restoration sites, but instead used approximately five times the standard seeding rate for each seed mix treatment type. High seeding rates are often used to mitigate potential effects of seed viability and herbivory on seedling establishment and increase overall plant densities (Applestein et al. 2018).

2.5.2. Plant Community Structure Following Restoration

We compared species richness following restoration with seed mixes containing a single source per species or multiple sources per species across two restoration sites. Multi-source seed mixes were associated with greater seeded species richness at the RSC site, but not the ORD restoration site. In the first growing season following the restoration four times the number of non-seeded species were observed compared to seeded species at the ORD site, and seven times at the RSC site, respectively (Figure 2.2). This is consistent with rates observed previously in grassland restoration experiments (Martin et al. 2005). Seeded species that emerged were those have evolved traits that provide competitive advantages in grassland ecosystems, such as rhizomatous root systems (Mangan et al. 2011; Dickson & Busby 2009) or mutualist fungal relationships which can promote and facilitate establishment (Busby et al. 2011). For example, *H. maximiliani* is a widespread perennial forb native to prairies in the United States and Canada (USDA). *H. maximiliani* readily established at both sites across seed treatments and is often found in remnant and restored prairies as a sub-dominant or dominant species (Dickson & Busby 2009). Previous studies have found that *H. maximiliani* is often one of the most productive forb species within plant communities as it may outcompete other species due to its rhizomatous root system that creates a spreading pattern for nutrient uptake, and thick sprouting stem that leads to

increased biomass production and vegetative coverage (McKenna et al. 2019; Mangan et al. 2011; Dickson & Busby 2009). *Ratibida columnifera* was another common perennial species to establish at the ORD site and across various seed treatments. This species occurs widely throughout southern Canada, across the US Great Plains, and into Northern Mexico (USDA). In previous experiments, *R. columnifera* has been observed to have high first year survival and a life span around three years and may negatively impact the abundance of other forbs (Lauenroth & Adler 2008; Dickson & Busby 2009). The competitive advantage expressed by *R. columnifera* may be due to its establishment through a prominent taproot and strong positive relationship with arbuscular mycorrhizal fungi which aids nutrient uptake and growth (Busby et al. 2011). In addition, both species are native to our study regions, thus may exist within the seed bank currently. However, during field site visits we did not observe *H. maximiliani* at either site outside of the experimental plots. *Ratibida columnifera* was present within the RSC site but was not included in the experimental seed mixes and was not present within the plots. Evaluating what species readily establish during the early stage of a restoration may aid in future seed mix design choices to combat non-native species establishment, and to ensure early restoration success.

Both the PERMANOVA and NMDS analyses assessed plant community structure using measures of diversity from seeded and non-seeded species quantified across seed mix treatments for each site. For RSC, the seed treatment with the most diverse community established throughout the season was our multiple-source mix (ABCDE). The multi-source seed treatments were planted on the portion of the site that was once a golf course, near a remnant mesic area with surrounding woody vegetation. Several species that established solely within this treatment were persistent within the woody vegetation nearby, including *Achillea millefolium*, *Plantago*

major, *P. annua*, and *Salix interior*. The presence of these species only within our multiple-source treatment plots is therefore likely influenced by the neighboring community, although as predicted this treatment had the most seeded species establish. This treatment was significantly different from all other seed mix treatments, except 'E' which was compositionally more similar during later seasonal months (Table A7). Given the spatial proximity of the 'ABCDE' and 'E' treatments, similar communities likely arose due to local site conditions, including below ground nutrient resources and varying seed banks across the site. Community composition at RSC also varied over time in response to seed mix treatments (Table 2). However, the spatial differences observed in community composition were maintained throughout the growing season.

Although multi-source seed mixes were associated with greater sown species richness within the RSC plots, total sown species richness was greater across all ORD plots, but not different across seed treatments (Figure 2.2). The increase in total seeded species richness could indicate there was less competition from non-seeded species which may allow for increased establishment, or seeded species already existed within the soil seed banks. Although seed treatment did not appear to influence sown species establishment within ORD plots, growing season influenced communities with similar community diversity establishing throughout the growing season (Figure 2.3B). Pairwise comparisons of community diversity across time indicated that June was the only month that was significantly different from the community present in later months. This may indicate that early season emergence drives the formation of community structure across time. These data provide a baseline understanding of site-specific community diversity to monitor composition change over time and across seed treatments.

Comparison across sites suggests the different patterns of diversity and those factors that structure diversity across sites are likely associated with different land-use histories. The

experimental seed mix treatments at the ORD restoration site were established on an old agricultural field with active management for hay production. The site has experienced similar land-use history, which has likely largely homogenized the above and belowground plant community, currently dominated by smooth brome (*B. inermis*) and alfalfa (*Medicago sativa*). The influence of agricultural activity and dominance of smooth brome and alfalfa has also likely contributed to further homogenization of the associated seed bank, reducing richness and diversity of the non-seeded species community (Bekker et al. 1997). In contrast to the homogeneity observed at the ORD site, the land-use history at RSC was more heterogeneous, which may have contributed to spatial variation in plant community establishment across the site. Interestingly, while the ORD community structure did not exhibit differences associated with seed mix treatment, the RSC site did exhibit significant differences across seed mix treatments. Single-source seed treatments A, B, and C were established on a portion of the site that was once planted with brome and alfalfa for haying purposes. In contrast, seed treatments D, E, and the multiple-source mix ABCDE were established on a portion of the site that was a golf course up until 2015. Combined, land use history and varying impacts of the seed bank and nutrient profile across the site suggests there is substantial heterogeneity across the site that may have influenced emergence following application of seed treatments. Despite site preparation methods used to prevent non-seeded species establishing within plots these differences may be reflected in the site-level differences as opposed to seed mix application. Thus site-level differences are due to spatial heterogeneity within the soil seed bank and nutrient availability associated with land-use history impacting community establishment regardless of seed mix treatment.

Land use history can play an important role influencing how restoration communities establish over time (Cousins et al. 2009; Grman et al. 2013). Spatial heterogeneity across a

restoration site could influence soil nutrient resources across the site and the associated species that may persist within the seed bank (Ricklefs 1977; Bakker et al. 2003). Where greater nutrient loading is observed, increased competition and exclusion between seeded and non-seeded species for resources could be observed (Eskelinen et al. 2021; Stotz et al. 2019). Aggressive non-seeded species often outcompete natives along nutrient load gradients leading to a subsequent loss of available soil nutrient resources. This can have substantial impacts to native plant diversity both above and belowground (Stevens & Carson 2002; Wilson & Tilman 1993; Eskelinen et al. 2021). Thus, heterogeneity in the soil nutrients or lack thereof likely impacted how communities established at both sites, but data on emergence provide a baseline to monitor how patterns in community composition may change over time.

Although we were interested in which seeded species established within our seed mix treatments, non-seeded species may also be important components to consider when evaluating these experimental communities over time. In a previous study Kaul & Wilsey (2020) noted that non-seeded weedy species abundance was the strongest predictor of species richness and diversity in grassland restorations, regardless of the age of the restoration. The most common non-seeded species to establish within our communities were introduced species, including cool-season grasses *B. inermis* and *Poa pratensis*. These species typically outcompete natives for resources, including both nutrient and light availability (reviewed in D'Antonio & Meyerson 2002). *Poa pratensis* establishes early in the spring before many native forbs, thus early establishment and the consequent increased growing season may provide a competitive advantage over native species (DeKeyser et al. 2015). *Bromus inermis* also establishes readily in the spring and is a commonly planted pasture grass that readily forms a quickly establishing monoculture through a rhizomatous root system (Stotz et al. 2019). The aggressive establishment

of *B. inermis* often leads to outcompeting and displacing native species which may lead to decreased plant diversity and community homogenization of a site when it becomes an established invader (Stotz et al. 2019; DiAllesandro et al. 2013). The prevalence of these well-known invasive species within our treatments, despite our pre-seeding site prep to limit non-seeded species establishment may indicate that more work is needed to successfully limit and manage their establishment during restoration. Considering how these species establish may be critical to restoration success as it may require more effort to shift these communities back to native species (Martin & Wilsey 2014). Additionally, genetic variation within seeded species used within seed mixes may mitigate some of the negative impacts of invasives. Genetic variation may increase the diversity of genotypes that establish increasing the probabilities of producing a self-sustaining, persistent population that can evolve over generations. Evaluating which non-seeded species establish and tracking their abundance in the early stages of a restoration will help guide restoration expectations and community management practices over time.

Single versus multiple source seed mix treatments did not have an impact on community composition diversity in the first year of restoration establishment. Our results suggest that early emergence and diversity within a plant community following restoration is largely influenced by land-use history. In addition, first-year emergence following restoration may be largely insensitive to seed mix type if non-seeded species in the seedbank are able to outcompete seeded species during establishment. Previous studies have shown that first year emergence positively influences seeded species abundance and richness several years following restoration (Applestein et al. 2018; Geaumont et al. 2019). Thus, while there is some evidence to suggest seed mix type may impact the diversity of established species, long-term assessments over multiple years will

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be necessary to quantify the full impact of seed mix type has to community diversity and restoration success over time. Evaluating what seeded and non-seeded species establish in the first year of a restoration will help inform future restoration plans for long-term restoration success. Indeed, identifying those seeded species that may have the competitive ability to readily establish may be needed during the design of seed mixes, both identifying those species that should be included and the proportion of seed that may be necessary to maintain those species over time.

2.6. Conclusions

Understanding the role within and among population genetic variation has on native grassland restorations may have substantial implications to seed mix design recommendations. We assumed here that a multi-population seed mix reflects increased genetic variation; however, the degree to which population sources impact standing genetic variation within seed mixes remains to be tested. Future work should include a genetic analysis of populations in single and multi-source seed mixtures to quantify genetic variation among the seed sources. Finally, although initial establishment results may be important to early restoration success, longer-term monitoring will be necessary to evaluate the impact seed mix treatment may have to community structure over time. Combined, genetic analysis and longer-term monitoring of seed mix treatments will provide information needed for land managers to establish seed sourcing guidelines critical to restoration in a changing environment.

2.7. Future Directions

Second year data was collected at both experimental restoration sites and will be incorporated into analyses. This data may be useful to track how community composition and diversity changes across time and may tease apart potential effects of seed mix treatment may

have on plant establishment. We may also evaluate how land-use histories may influence restoration community across multiple years.

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3. MODELING HABITAT SUITABILITY FOR 26 COMMON GRASSLAND RESTORATION PLANT SPECIES UNDER TWO CARBON EMISSION CLIMATE CHANGE SCENARIOS

3.1. Abstract

Understanding how climate change may impact the presence of species across heterogeneous landscapes is needed to inform both conservation and restoration priorities within critically imperiled grassland systems. The relationship between species presence across diverse landscapes and the climatic factors associate with those landscapes can be used to predict species' ecological niche. These relationships may then be used to quantify the expected distribution for species under contemporary climates in addition to predicting how that distribution may be altered under climate change projections. Species Distribution Modeling (SDM) approaches are widely used to model the ecological niche and associated geographic distribution of species of concern. We selected 26 grassland species commonly used in restoration across six functional-groups, including: asters, cool-season grasses, legumes, early-blooming forbs, late-blooming forb, and warm-season grasses to model habitat suitability across contemporary and future climates. We first identified key climate factors that were correlated with species presence across their distribution and using these climatic factors modeled habitat suitability for individual species considering contemporary climates and those projected under low and high carbon dioxide emission scenarios. This study indicates that although many grassland species coexist in the same habitat, the climate factors that influence species presence across the landscape are highly variable.

For functional groups, models for legumes and late-blooming forbs predicted limited change in habitat suitability in response to changing carbon emissions. For restoration seed

mixes, these functional groups may be reliable indicators of climate response due to their conserved responses. However, most functional groups had high variability in habitat change across scenarios. Warm-season grasses showed significantly larger ranges habitat suitability expansion and contraction at range margins. This indicates that it may be useful to identify specific species within some functional groups for restoration seed-mix purposes over community assembly approaches. These data inform our understanding of contemporary and potential future niche for common grassland species and can be used to identify regions within a distribution that remain highly suitable under current conditions for potential seed sourcing and identify regions appropriate for restoration for species under future climate scenarios.

3.2. Introduction

Species distributions are influenced by multiple factors, including dispersal mechanisms that enable colonization of suitable habitat (Soberon & Peterson 2005; Hargreaves & Eckert 2014), natural or artificial boundaries that influence dispersal potential and connectivity (Sheth et al. 2020), inter- and intra- specific competitive interactions of species (Soberón & Nakamura 2009), and local environmental conditions (Hutchinson 1957; Pearson & Dawson 2003; Araújo & Peterson 2012). For many species, environmental conditions are key factors contributing to species' ability to persist across broad geographic distributions (Sheth et al. 2020). Indeed, the relationship between species' presence and climatic factors underlying those presence points can be used broadly to understand species' ecological niche (Araújo & Pearson 2005; Bradie & Leung 2017; Kamyo & Asanok 2020). This relationship assumes that one of the greatest limiting factors influencing a species' ability to persist across diverse landscapes is local abiotic conditions, although there is substantial evidence that this is the case (Sexton et al. 2009; Pearson & Dawson 2003; Hargreaves et al. 2014). Understanding those climate factors that interact to

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influence species presence across landscapes can be used to understand species contemporary distributions, and predict potential range shifts in response to environmental change (Hargreaves et al. 2014; Pearson & Dawson 2003). This is particularly important in a restoration and conservation context as these data can be used to identify important areas for seed sourcing or identifying regions where restoration may be suitable under current and future climate scenarios.

Species distribution models (SDMs) are powerful tools that can be used to understand and visualize species' relationship with climate across diverse landscapes (Kearney & Porter 2009; Guisan & Zimmermann 2000). These models have become standard in many fields due to their wide-scale applicability and statistical tests that can be used to model species' ecological niche (Franklin 2013; Peterson & Soberón 2012). SDMs typically use species' geospatial presence data and associated environmental layers to create predictive maps of species' distributions (Soberón & Nakamura 2009; Elith & Leathwick 2009). Presence data may include both contemporary field observations or online databases such as Global Biodiversity Information Facility (GBIF) which provide occurrence data associated with herbarium collections, scholarly institutes worldwide, and citizen science observations. These databases provide rapidly accessible datasets for many species across time and are continuously updated making these data a powerful tool to aid in modeling for management or conservation decisions (Newbold 2010; Anderson et al. 2016). For example, SDMs have been used to identify habitat translocation suitability for species of conservation concern (Guisan et al. 2013; Eyre et al. 2022), to estimate the effects of climate change on habitat suitability over time (Garcia et al. 2013; Ramírez-Preciado et al. 2019), or identify invasion risk by exotic species (Thuiller et al. 2005; Peeler & Smithwick 2018). In this study we will use SDMs to predict current habitat

suitability of 26 common grassland species and evaluate how suitability changes across climate change scenarios in the future.

The ease of use of these tools, however, also means that the decisions used in creating SDMs need to be carefully considered in order to ensure biologically meaningful relationships are modeled (Wiens et al. 2009). For example, spatial bias in presence points may skew how climate relationships are estimated within models (Phillips et al. 2009). In addition, climate variables that are incorporated into models used in forecasting need to be biologically relevant predictors for modeled species to accurately predict the climate niche (Elith & Leathwick 2009). Although there are caveats to using climate data to model species' niches, previous studies have shown that climate models can be more predictively powerful than models that use other data including biotic interactions (Araújo & Luoto 2007; Austin & Van Niel 2011). High predictive accuracy for contemporary models using solely climate data suggests that they may be reasonable models to use to predict changes to the ecological niche under climate change (Araújo & Luoto 2007). For restoration and conservation these data can be used to identify regions to source seed from to use in ecosystems of conservation concern (Havens et al. 2015; Harrison et al. 2017). Additionally, these models will be crucial to identifying climactically suitable habitat in response to climate change scenarios.

Temperate grasslands are one of the most critically imperiled ecosystems globally. The loss of grasslands due to anthropogenic conversion has motivated extensive restoration and conservation efforts to maintain essential ecosystem services and biodiversity (Hoekstra et al., 2005, Comer et al. 2018). Restoration often relies on historical information to restore grasslands to a reference state (Miller & Hobbs 2007) and to maintain biodiversity (Montoya et al. 2012; Barr et al. 2017). More recent approaches have suggested combining historic information with

evidence from ongoing restoration and empirical research is needed to ensure restorations are successful long-term in response to environmental change (Perring et al. 2015; Perkins et al. 2019; Gann et al. 2019). In addition, a critical component to grassland restoration is to identify which species to use in seed mixes, and where to source seed for establishing seed mixes (Broadhurst et al. 2008). Generally, increasing species richness within restorations is associated with increased productivity needed for long-term persistence (Tilman et al. 2001, 1996). Using species that span multiple functional groups may allow restorations to fill ecological niche space following restoration or may allow a restoration community to compete successfully against invasive species with similar functional traits (Oakley & Knox 2013; Norland et al. 2013). Ideally, plant species considered for restoration purposes would be able to thrive under both current and projected climate for the site. Climate projections for the Northern Great Plains region indicate an increase in extreme climactic events, such as precipitation and heat wave events that may occur with high degrees of variability (Kluck et al. 2018). Changes in temperature and precipitation may alter blooming phenology and reproductive timing of some grassland species (Dunnell & Travers 2011; Whittington et al. 2015); however, the extent of these shifts is likely to vary (Cook et al. 2012; Chandler & Travers 2021). Along with changing temperature and precipitation events, atmospheric carbon dioxide (CO_2) emissions may impact how different plant functional groups establish and persist (Polley et al. 2013; Kluck et al. 2018). Modeling current and future climatic niches provide a way to evaluate how species ranges may change in response to changing environmental conditions. Using these results, restoration practitioners can optimize species to include within a seed mix and ensure seed is collected from regions of high suitability now and into the future.

In this study we aimed to (1) identify climate factors related to seasonal variation in precipitation and temperature that influence the presence of 26 grassland species commonly used in restoration throughout the Great Plains, (2) map the contemporary climatic niche for each species across the entirety of its range, and (3) use the understanding of the contemporary climatic niche to predict habitat suitability in the future under both low and high-carbon emission predictions. Results of this study may be used to identify where suitability of habitat may be maintained in response to changing climate scenarios and where suitable habitats may be lost. These results will be used to guide identification of species to use in seed mixes, identification of potential regions to source seed, and potential restoration sites in the future.

3.3. Methods

3.3.1. Data Collection and Preparation

During the summer of 2019, populations of 26 common grassland forb and grass species (Table 1) were mapped using ESRI's Collector application (ESRI 2019) on remnant grasslands across Minnesota, North Dakota, and South Dakota USA. These 26 species were chosen due to their substantial geographic distribution and common use in grassland restorations and represent a variety of functional groups necessary to maintain biodiverse and productive grasslands (Table 3.2.1; Smith 2010; Dixon et al. 2017). These data were used as ground-truthed presence locations for use modelling species climatic niche through SDM techniques. To complement ground-truthed points, occurrence records for each species were extracted from the Global Biodiversity Information Facility database (GBIF; 05 September 2020; www.gbif.org). These data compile citizen science observations and herbarium records to create a contemporary global record for species' geographic distribution. Records were filtered to include only those occurrences with geo-coordinates available, as necessary to correlate climate conditions at the geo-spatial location

of presences. Following this, observations that were recorded prior to the year 1900 were excluded to ensure any false or imprecise collections were removed. Records with over 1 km uncertainty were removed to ensure accuracy on the landscape. To correct for potential data entry errors from outlier points that occurred outside of known species ranges, remaining observations were visually filtered and were removed if necessary. Lastly, we removed any GBIF records intersecting with our ground-truth mapped locations points. By combining field-collected presence data with online database records not only provides a way to ensure a full species range is captured, but also ensures that SDM algorithms can maintain default settings to ensure the most suitable model complexity is retained for accurate measures of predictive accuracy (Syfert et al. 2013).

Table 3.1. Individually modeled grassland species used in current and future modeling scenarios. Broad functional group for each species listed where Forb - EB and LB represent early and late blooming forbs respectively. Grass CS and WS represent cool season and warm season grasses respectively. Number of presence records used in modeling includes mapped presence points and GBIF record data after quality control filtering process to create a final dataset used in model training and evaluation.

Species Scientific	Species Common	Species Code	Functional Group	No. of presences
Amorpha canescens	Leadplant	AMOCAN	Legume	1,991
Anemone cylindrica	Thimbleweed	ANECYL	Forb - EB	1,109
Artemisia frigida	Fringed Sage	ARTFRI	Aster	1,507
Asclepias speciosa	Showy Milkweed	ASCSPE	Forb - LB	2,405
Bouteloua curtipendula	Side-oats grama	BOUCUR	Grass - WS	1,913
Bouteloua gracilis	Blue grama	BOUGRA	Grass - WS	1,420
Dale purpurea	Purple Prairie Clover	DALPUR	Legume	1,928
Echinacea angustifolia	Narrow-leaf Coneflower	ECHANG	Aster	126
Geum triflorum	Prairie Smoke	GEUTRI	Forb - EB	2,084
Helianthus maximiliani	Maximilian Sunflower	HELMAX	Aster	1,386
Helianthus pauciflorus	Stiff Sunflower	HELPAU	Aster	967
Hesperostipa comata	Needle and thread	HESCOM	Grass - CS	826
Hesperostipa spartea	Porcupine grass	HESSPA	Grass - CS	1,043
Koeleria macrantha	June Grass	KOEMAC	Grass - CS	1,584
Liatris aspera	Rough Blazingstar	LIAASP	Aster	1,067
Liatris punctata	Dotted Blazing Star	LIAPUN	Aster	1,356
Pediomelum argophyllum	Silverleaf Scurfpea	PEDARG	Legume	1,265
Penstemon gradiflorus	Large Beardtongue	PENGRA	Forb - EB	478
Potentilla arguta	Prairie Cinquefoil	POTARG	Forb - EB	1,010
Ratibida columnifera	Prairie Coneflower	RATCOL	Aster	4,288
Rudbeckia hirta	Black-eyed Susan	RUDHIR	Aster	6,087
Schizachyrium scoparium	Little Bluestem	SCHSCO	Grass - WS	2,499
Solidago rigida	Stiff Goldenrod	SOLRIG	Aster	1,567
Symphyotrichum novae-angliae	New England Aster	SYMNOV	Aster	2,424
Verbena hastata	Blue Vervain	VERHAS	Forb - LB	2,641
Vebena stricta	Hoary Vervain	VEBSTR	Forb - LB	1,610

3.3.2. Predictor Variable Selection

Climate data was downloaded from WorldClim, which includes 19 'bioclimatic' variables relating to seasonal temperature and precipitation patterns (http://www.worldclim.org/) using version 2.1 at 2.5 arc-minute (~5 km) resolution within the GFDL-ESM4 Global Circulation Model (GCM) (Fick & Hijmans 2017). Fine-scale resolution of gridded climate data

captures variation in environmental gradients (Fick & Hijmans 2017). Using the appropriate climate variables relevant for individual species modeling is a critical consideration as species may not be influenced by the same environmental conditions (Pearson & Dawson 2003). It is important to identify which climate variables are ecologically relevant to predict species distributions as different species may be constrained by different environmental conditions (Ashcroft et al. 2011). This is particularly true for species with broad climatic niches. Consequently, variable selection was used to estimate factors contributing to species ecological niche spaces (Beaumont et al. 2005). To predict contemporary distributions, we initially used all 19 bioclimatic variables as potential predictor variables using near-current climate data (1970-2000; Table 3.1) to evaluate which variables were important for predicting habitat suitability for each individual species. Climate predictors that contributed to the model from the most to the least were retained until cumulatively 90% of model contribution was explained by the predictor variables (Figure 3.2). These variables were retained as they are predicted to be the most important to describing an individual species' ecological niche space (Bradie & Leung 2017; Ramasay et al. 2021). In addition, we used the 'vifstep' (usdm package; Naimi 2017) to compare another variable selection procedure against our selected climate variables to ensure only the most important variables were selected per species. The method uses a stepwise procedure removing variables to ultimately exclude highly collinear variables which may be redundant in modeling (Naimi 2017). Once climate variable selection was complete, contemporary habitat suitability models based on near-current climate were produced for each species. These climatic variables were used to model species' distributions associated with future climates assuming that the same climactic variables that influence species presence on the landscape currently will be the same in the future.

Table 3.2. BIOCLIM variables of climate predictors and descriptions used in MaxEnt model calibration and final predictions of habitat suitability for contemporary and future species models. Data available from Worldclim.org.

Variable Number	Variable Description
BIO 1	Annual Mean Temperature
BIO 2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO 3	Isothermality (BIO2/BIO7) ($\times$100)
BIO 4	Temperature Seasonality (standard deviation $\times$100)
BIO 5	Max Temperature of Warmest Month
BIO 6	Min Temperature of Coldest Month
BIO 7	Temperature Annual Range (BIO5-BIO6)
BIO 8	Mean Temperature of Wettest Quarter
BIO 9	Mean Temperature of Driest Quarter
BIO 10	Mean Temperature of Warmest Quarter
BIO 11	Mean Temperature of Coldest Quarter
BIO 12	Annual Precipitation
BIO 13	Precipitation of Wettest Month
BIO 14	Precipitation of Driest Month
BIO 15	Precipitation Seasonality (Coefficient of Variation)
BIO 16	Precipitation of Wettest Quarter
BIO 17	Precipitation of Driest Quarter
BIO 18	Precipitation of Warmest Quarter
BIO 19	Precipitation of Coldest Quarter
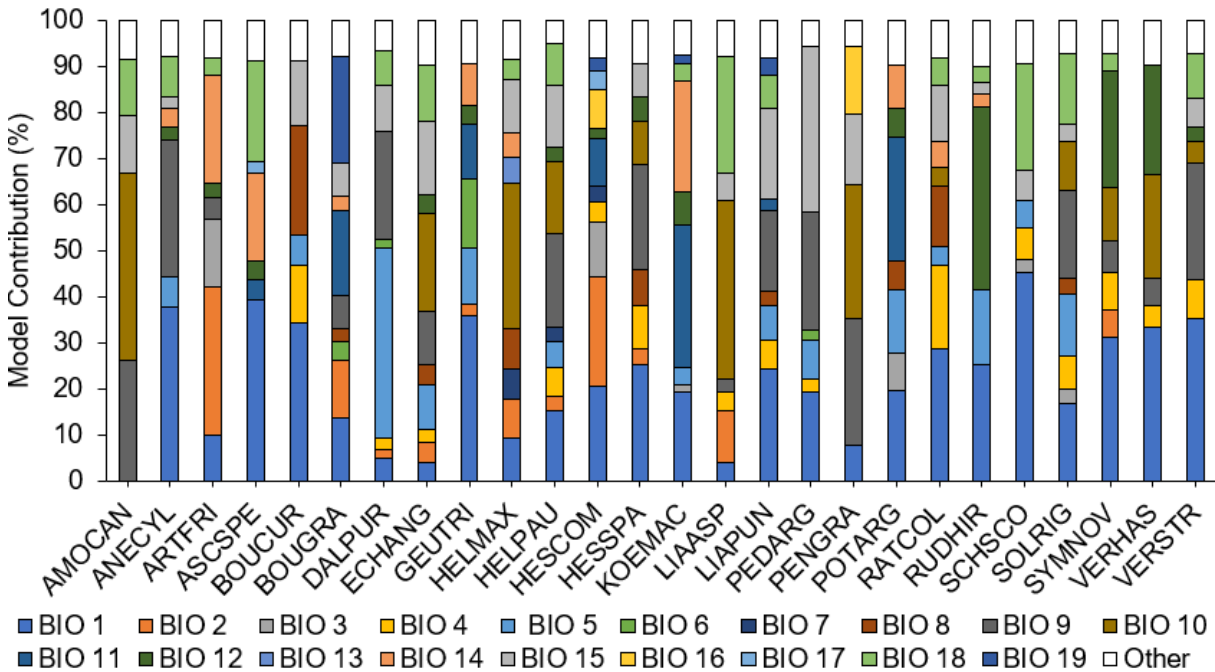
Figure 3.1. Individual variable contributions to MaxEnt models run using all 19 bioclimatic environmental variables for individual species modeled listed by species code. Variables that contributed the most to the individual species modeling were retained up to a cutoff of 90 for near current and future climate scenarios. White bars labeled "Other" represent all other variables that were not retained for further modeling.

3.3.3. Species Distribution Modeling

Climate data for each BIOCLIM variable used in modeling was extracted for all presence points, ground-truthed points and GBIF occurrence records. Presence point climate data is essential for habitat modeling as these values represent the realized climate associated with a species occurrence. These data collectively represent the climate niche across a species' entire range. Presence point data should be equally sampled across a species' entire range to reduce sampling bias in the model; however it is often difficult to accurately capture equal representation across a species' entire range (Guisan & Zimmermann 2000). Presence data is typically considered biased as most data is collected in a localized area where surveys occur or where herbarium records are collected (Phillips et al. 2009; Syfert et al. 2013). Spatial filtering is one way to minimize spatial bias in modeling, and involves removing data that is typically

collected within a set distance relevant to species' dispersal or geographic boundary (Boria et al. 2014). This method may reduce the total amount of presence points used per species, and may reduce MaxEnt model performance when sample sizes are low; therefore, accounting for sampling bias in background data is a preferred approach (Guisan & Zimmermann 2000; Syfert et al. 2013). Background data is used commonly within MaxEnt modeling as MaxEnt uses presence-only data, meaning true absences of a species are not known (Grimmett et al. 2020). Absence data is typically preferred in modeling as these data represent climate where individuals within a species do not exist and would theoretically represent the climate range that a species cannot exist within (Elith et al. 2011). Verifying absence data represents true absences and are not a result of sampling bias, however, is very difficult to do and as such background points are most often used (Phillips et al. 2009). These points are used within modeling to predict habitat suitability outside of known presence points. Previous work has shown spatial bias in background points increases the chance models overpredict suitability where a species would likely be absent (Lobo et al. 2008; Kramer-Schadt et al. 2013). To account for sampling bias within the Northern Great Plains region where we sampled species presences in the field, we balanced background sampling to ensure points that would cluster within the region and across a species entire range to create a sample that was representative of all potential suitable climatic conditions for individual species (Kramer-Schadt et al. 2013; Galante et al. 2018). Five thousand background points were collected within a 50km radius from all ground-truth points. An additional 5,000 randomly generated background points were collected across the species' entire geographic range but did not intersect within known presence points nor the sampling bias background points. Once background points were established, climate data was extracted for modeling. Presence and background data was used with MaxEnt to model climate suitability for

each individual species. Models were run with standardized MaxEnt settings, with the beta-multiplier setting at 0.15 to adjust model complexity and reduce the chance of overfitting model predictions (Elith, Steven, & Phillips, et al. 2011). All species' distribution models were run in RStudio (version 4.0.2) using MaxEnt (version 3.4.1; Phillips et. al 2006; Elith et al. 2011).

3.3.4. Model Evaluation

To evaluate model performance, we performed a cross-validation. Each species' model was evaluated with a k-fold (k=5) data partitioning using 80% and 20% of our presence point data for training and testing models respectively (Elith et al. 2011). Presence points are randomly subset for training data of approximately equal sizes (k=4) to first fit the model and the remaining set is used as testing data (k=1) to evaluate the MaxEnt model's fit to the data, and to ensure predictive accuracy is maintained to validate model choice (Elith & Leathwick 2009; Elith et al. 2011). Partitioning species presence data into independent data sets is a common way to evaluate a model's predictive capacity but requires a dataset with enough presence pointes for a model to discriminate between true presence and absence on the landscape (Guisan et al. 2006). All individual species modeled had sufficient presence records used for data partitioning and final model testing (Syfert et al. 2013). The average number of presence records across species was 1,792 (478 – 6,087; Table 3.2.1).

Model robustness, defined as a model's ability to correctly identify true species presences and absences, was evaluated using Maxent's standardized optimization algorithm using 10,000 background points, and 500 iterations (Braunisch et al. 2013). For each model, the associated area under the receiver-operator characteristic curve (AUC) was used as a common threshold to indicate the model's predictive accuracy (Elith & Leathwick 2009; Merow et al. 2013). Higher AUCs indicate a well-fit model that can successfully discriminate true presence and absences on

the landscape, better than a null model (Guisan et al. 2006). Typical models use an acceptable

AUC cut off score of >0.75, where a null fit model scores 0.5 (Jiménez-Valverde 2012; Merow

et al. 2013). Although there is some criticism of using the AUC as a method of model evaluation

due to possible value inflation, where models overpredict presences (see Lobo et al. 2008;

Jiménez-Valverde et al. 2008); our protocol followed methods designed to account for common

errors in models that inflate predictive accuracy. We accounted for potential bias within

occurrence records, we used background points that accounted for both spatial bias within our

sampling region and within the entire species range distribution, and our climate predictor

variables were restricted to only the variables with the most model contribution and underwent a

secondary test for collinearity. This procedure was similarly followed by Lyon et al. (2019), and

thus potential modeling errors were accounted for to ensure AUC values were not inflated.

Following model validation, final models were established to estimate the contemporary

distribution for each of the 26 species. These models included all presence data, no longer split

into training and testing data sets, and used the same BIOCLIM variables and MaxEnt

parameters used in the training and testing sets. AUC scores were assessed for each final model

to ensure predictive accuracy was maintained. Final models produced species distributions maps

as a binary raster from 0 to 1 where 0 indicates low probability of presence and 1 indicates a high

probability of presence. Raster layers were imported into ArcGIS Pro (ESRI; version 2.8 & 2.9)

and species distributions were reclassified into four classes of habitat suitability; No suitability

(0–0.2), Low suitability (0.2–0.4), Moderate suitability (0.4–0.6), and High suitability (0.6–1)

based on Zhang et al. 2018 and Ramasamy et al. 2021.

3.3.5. Climate Change Scenario Modeling

Future habitat suitability (2021-2040) was evaluated for each study species using two future climate change scenarios available from WorldClim and built off phase 6 of the Coupled Model Intercomparison Project (CMIP6) climate change projections and Shared Socio-economic Pathways (SSP). CMIP6 models produced more realistic expectations of future habitat suitability as these models were produced with the most recent data on greenhouse gas emissions and concentrations from the Sixth Assessment report from the Intergovernmental Panel on Climate Change (IPCC). At the time of analysis, WorldClim only offered future climate data on select scenarios with limited spatial resolution, which limited the data availability in the WorldClim database and the future scenarios available. Thus, we evaluated habitat suitability in the future the "low" (SSP 126) and "high" (SSP 370) carbon emission scenarios. Both scenarios use projections of greenhouse gas concentrations and emissions but vary in the intensity of emissions. The low carbon emission scenario lies within the "sustainability" track that aims to reach the Paris Agreement climate conditions (Meinshausen et al. 2020). The high carbon emission scenario acts as a "business as normal" model that has medium-high rates of concentrations and emissions (Meinshausen et al. 2020). Future models were evaluated with the same environmental predictors identified using near current climate data and the same MaxEnt model protocol. Future habitat was classified and calculated as habitat that becomes 1) more suitable (or growth), 2) unsuitable (or reduction), and 3) unchanged in suitability, and were illustrated in ArcGIS Pro accordingly following similar protocol to (Zhang et al. 2018). Habitat change for growth and loss was calculated as the number of pixels within each category multiplied by the total number of pixels for each species raster file to estimate area change in

km^2. We then evaluated habitat change based on functional groups to identify any patterns within groups and across the climate change scenarios.

3.4. Results

3.4.1. Climate Variable Selection

Climate variables identified as most important to predicting habitat suitability for grassland species varied widely across the 26 species. However, of the 19 climate variables assessed, BIO 1 (Mean Annual Temperature), BIO 4 (Temperature Seasonality), BIO 5 (Max Temperature of the Warmest Month), BIO 9 (Mean Temperature of the Driest Quarter), BIO 10 (Mean Temperature of the Warmest Quarter), BIO 12 (Annual Precipitation), BIO 15 (Precipitation Seasonality), and BIO 18 (Precipitation of the Warmest Quarter) were identified consistently as important and were commonly retained for over half of the species modeled (Figure 3.3; Table 3.3). BIO 1 contributed substantially to every species model except *Amorpha canescens* (Leadplant). The least commonly retained climate variable was BIO 13 (Precipitation of Wettest Month) which was used only once for *Helianthus maximiliani* (Maximilian Sunflower).

Table 3.3. Species used in modeling listed by species code and bioclimactic variables from WORLDCLIM that were retained in current and future habitat suitability modeling.

Species Code	Variables Retained for Modeling						
AMOCAN	BIO 4	BIO 9	BIO 10	BIO 15	BIO 18	-	-
ANECYL	BIO 1	BIO 5	BIO 9	BIO 12	BIO 14	BIO 15	BIO 18
ARTFRI	BIO 1	BIO 2	BIO 3	BIO 6	BIO 9	BIO 12	BIO 14
ASCSPE	BIO 1	BIO 11	BIO 12	BIO 14	BIO 17	BIO 18	-
BOUCUR	BIO 1	BIO 4	BIO 5	BIO 8	BIO 15	-	-
BOUGRA	BIO 1	BIO 2	BIO 6	BIO 8	BIO 9	BIO 11	BIO 14
DALPUR	BIO 1	BIO 2	BIO 4	BIO 5	BIO 6	BIO 9	BIO 15
ECHANG	BIO 1	BIO 2	BIO 4	BIO 5	BIO 8	BIO 9	BIO 10
GEUTRI	BIO 1	BIO 2	BIO 5	BIO 6	BIO 11	BIO 12	BIO 14
HELMAX	BIO 1	BIO 2	BIO 7	BIO 8	BIO 10	BIO 13	BIO 14
HELPAU	BIO 1	BIO 2	BIO 4	BIO 5	BIO 7	BIO 9	BIO 10
HESCOM	BIO 1	BIO 2	BIO 3	BIO 4	BIO 7	BIO 11	BIO 12
HESSPA	BIO 1	BIO 2	BIO 4	BIO 8	BIO 9	BIO 10	BIO 12
KOEMAC	BIO 1	BIO 3	BIO 5	BIO 11	BIO 12	BIO 14	BIO 18
LIAASP	BIO 1	BIO 2	BIO 4	BIO 9	BIO 10	BIO 15	BIO 18
LIAPUN	BIO 1	BIO 4	BIO 5	BIO 9	BIO 11	BIO 15	BIO 18
PEDARG	BIO 1	BIO 4	BIO 5	BIO 6	BIO 9	BIO 15	-
PENGRA	BIO 1	BIO 9	BIO 10	BIO 15	BIO 16	-	-
POTARG	BIO 1	BIO 3	BIO 5	BIO 8	BIO 11	BIO 12	BIO 14
RATCOL	BIO 1	BIO 4	BIO 5	BIO 8	BIO 10	BIO 14	BIO 15
RUDHIR	BIO 1	BIO 5	BIO 12	BIO 14	BIO 15	BIO 18	-
SCHSCO	BIO 1	BIO 3	BIO 4	BIO 5	BIO 15	BIO 18	-
SOLRIG	BIO 1	BIO 3	BIO 4	BIO 5	BIO 8	BIO 9	BIO 10
SYMNOV	BIO 1	BIO 2	BIO 4	BIO 9	BIO 10	BIO 12	BIO 18
VERSTR	BIO 1	BIO 4	BIO 9	BIO 10	BIO 12	-	-
VERHAS	BIO 1	BIO 4	BIO 9	BIO 10	BIO 12	BIO 15	BIO 18

3.4.2. Model Assessment

All models were successful at discriminating between unsuitable and suitable habitat with the five-fold training and test data indicating that models were well fit. High predictive accuracy and model fit were maintained in final models using all individual species presence data. The average training AUC across all species was 0.946, indicating high model accuracy. AUC values slightly decreased to an average of 0.913 for final models produced for habitat suitability with a

range of 0.789 – 0.969. Future climate predictions also had high predictive accuracy of 0.912 and

0.913, and ranges of 0.788 – 0.971 and 0.789 – 0.972 for SSP 126 and SSP 370 respectively

(Table 3.4).

Table 3.4. AUC values for training and testing models, contemporary, and under low carbon emissions scenarios (SSP 126) and high carbon emissions scenarios (SSP 370).

Species Code	AUC_training	AUC_contemporary	AUC_126	AUC_370
AMOCAN	0.971	0.936	0.935	0.934
ANECYL	0.972	0.943	0.944	0.944
ARTFRI	0.954	0.917	0.915	0.917
ASCSPE	0.929	0.88	0.88	0.884
BOUCUR	0.93	0.885	0.882	0.886
BOUGRA	0.94	0.901	0.898	0.898
DALPUR	0.953	0.908	0.907	0.907
ECHANG	0.977	0.959	0.96	0.958
GEUTRI	0.925	0.884	0.884	0.883
HELMAX	0.97	0.934	0.934	0.937
HELPAU	0.982	0.961	0.963	0.962
HESCOM	0.958	0.935	0.933	0.935
HESSPA	0.983	0.963	0.963	0.963
KOEMAC	0.907	0.875	0.876	0.873
LIAASP	0.968	0.948	0.946	0.945
LIAPUN	0.959	0.92	0.919	0.923
PEDARG	0.976	0.961	0.96	0.962
PENGRA	0.986	0.969	0.971	0.972
POTARG	0.946	0.915	0.914	0.914
RATCOL	0.925	0.861	0.862	0.861
RUDHIR	0.791	0.789	0.788	0.789
SCHSCO	0.909	0.86	0.857	0.86
SOLRIG	0.955	0.918	0.92	0.92
SYMNOV	0.953	0.905	0.906	0.904
VERHAS	0.93	0.874	0.876	0.875
VERSTR	0.954	0.927	0.929	0.929

3.4.3. Forecasting Habitat Suitability Under Climate Change

Changes in habitat suitability were evaluated at two levels, first by individual species

modeled and second by species' functional group. Habitat reduction was quantified as habitat

that was predicted as suitable in contemporary models and became less suitable in future models. Similarly, habitat suitability gain was quantified in predicted habitat suitability gains in the future compared to contemporary models. Ranges of habitat suitability reductions and gains varied widely across all species. Habitat suitability reductions varied between $245 - 1,471km^2$ and $168 - 1,523km^2$ between low and high carbon emission scenarios respectively (Table 3.5). Habitat suitability gains varied between $232 - 2,043km^2$ and $127 - 1,853km^2$ between low and high carbon emission scenarios respectively (Table 3.5). Interestingly, across scenarios *Bouteloua gracilis* (Blue grama) had the highest amount of predicted habitat suitability reduction and gain (Figure 3; Table 3).

Across functional groups, habitat loss and gain were also variable. In both low and high carbon emission scenarios, warm-season grasses had the highest amount of both habitat loss and gain (Fig 2). Late-blooming forbs and legumes displayed on average, lower amounts of both habitat loss and gain across both scenarios (Fig 2). Differences in predicted habitat suitability reduction and gain across the functional groups were first evaluated using a two-way analysis of variance (ANOVA) to account for the interaction of functional groups to carbon emission scenarios. The only significant effect in explaining differences in predicted habitat changes was by functional group, climate change scenario and the interaction between the two was not significant. As there was no significant interaction, we used one-way ANOVAs and Tukey-Kramer post-hoc analyses to determine which functional groups were significantly different for predicted habitat change for both habitat suitability reduction (classified as loss) and gain (classified as growth). The Tukey-Kramer post-hoc analysis was run using the unbalanced parameters setting to account for unequal replication (i.e. There are varying numbers of species classified for each different functional group). For the low carbon emission scenario late-

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blooming forbs and warm-season grasses had significant differences in the amount of habitat loss

different from the other functional groups and with each other (Figure 3.2A). For habitat growth

in the low carbon scenario, warm-season grasses and legumes were also significantly different

(Figure 3.2A). For both categories of habitat change, warm-season grasses had greater amounts

of area change within the low-carbon scenario. Although the total suitability was increasing for

warm-season grasses, the amount that was reduced was also higher than across other functional

groups. Across both scenarios, late-blooming forbs and legumes had relatively conservative

amounts of habitat change. Late-blooming forbs had significantly lower amounts of habitat loss

within the low carbon emissions scenario, and significantly lower amounts of habitat growth in

the high emissions scenario (Figure 3.2). Legumes had significantly lower habitat gains across

both carbon emission scenarios compared to other functional groups (Figure 3.2). For the high-

carbon emissions scenario, habitat changes were only significant based on habitat growth. There

were no differences between functional groups for habitat loss. Similar to the low-carbon

scenario, warm-season grasses had, on average, more habitat growth than the other functional

groups (Figure 3.2B). Asteraceae, late-flower forbs, legumes had, on average, lower amounts of

habitat growth (Figure 3.2B).

Table 3.5. Individually modeled grassland species used in near current (1970-2000) and future (2021-2040) habitat suitability modeling. Area loss and growth in kilometers for each species listed under low and high carbon emission climate scenarios. Broad functional group for each species listed where Forb - EB and LB represent early and late blooming forbs. Grass CS and WS represent cool season and warm season grasses.

| Species Scientific	Functional Group	Low carbon emission		High carbon emission	
		Area Loss (km^2)	Area Growth (km^2)	Area Loss (km^2)	Area Growth (km^2)
Amorpha canescens	Legume	418	232	492	197
Anemone cylindrica	Forb - EB	298	911	344	961
Artemisia frigida	Aster	367	652	688	445
Asclepias speciosa	Forb - LB	319	513	490	542
Bouteloua curtipendula	Grass - WS	506	746	290	942
Bouteloua gracilis	Grass - WS	1,471	2,043	1,526	1,853
Dale purpurea	Legume	435	459	450	595
Echinacea angustifolia	Aster	332	305	399	300
Geum triflorum	Forb - EB	394	444	645	483
Helianthus maximiliani	Aster	512	567	496	685
Helianthus pauciflorus	Aster	296	755	247	575
Hesperostipa comata	Grass - CS	782	441	797	567
Hesperostipa spartea	Grass - CS	782	441	797	567
Koeleria macrantha	Grass - CS	1,162	580	1,213	552
Liatris aspera	Aster	1,025	298	878	291
Liatris punctata	Aster	974	725	493	546
Pediomelum argophyllum	Legume	476	350	447	457
Penstemon gradiflorus	Forb - EB	328	350	168	543
Potentilla arguta	Forb - EB	829	991	715	1,443
Ratibida columnifera	Aster	559	1,132	519	1,156
Rudbeckia hirta	Aster	643	460	466	472
Schizachyrium scoparium	Grass - WS	1,311	786	1,267	1,030
Solidago rigida	Aster	955	955	1,061	721
Symphyotrichum novae-angliae	Aster	285	192	309	127
Vebena stricta	Forb - LB	245	463	354	527
Verbena hastata	Forb - LB	400	277	266	426
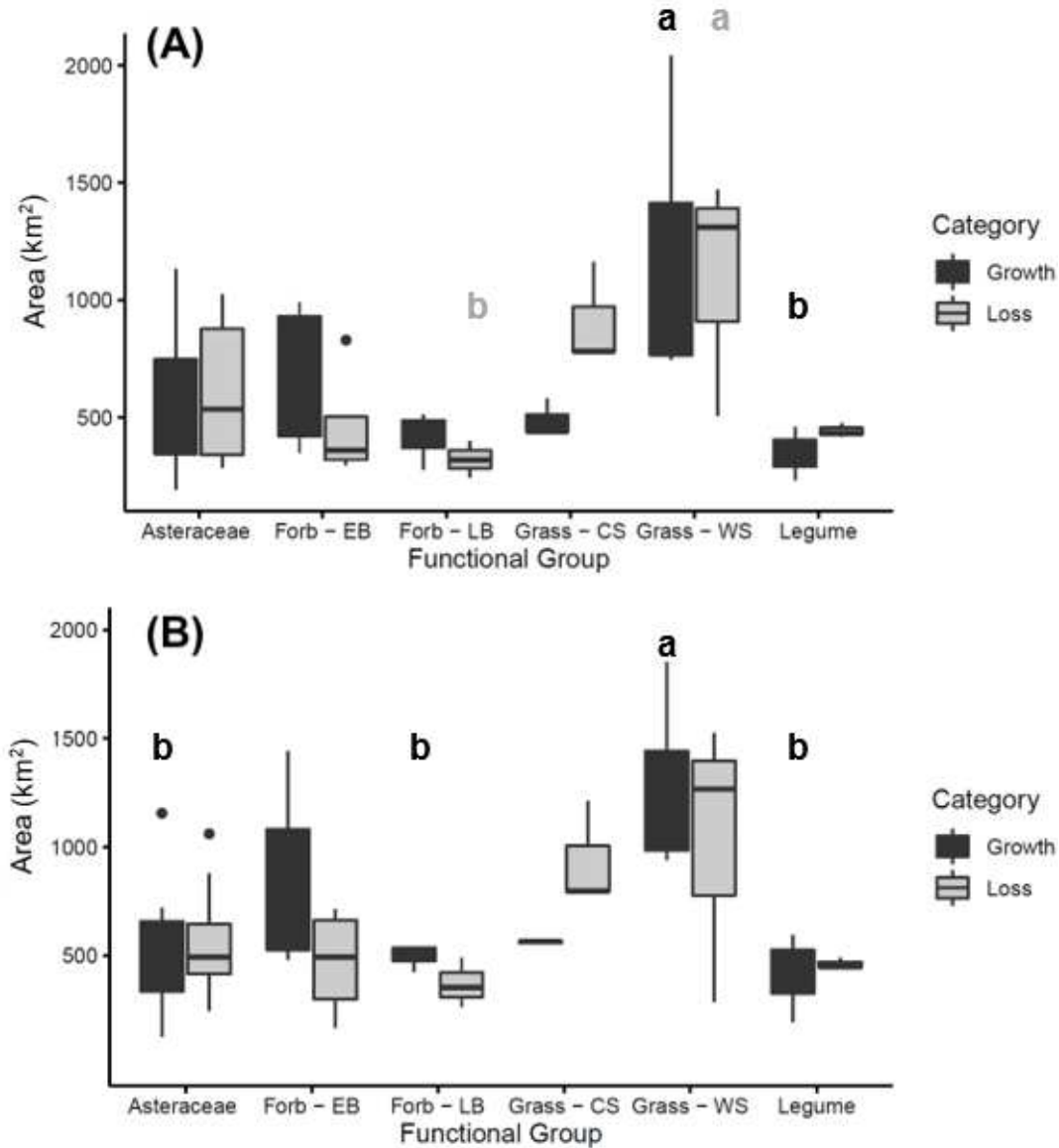
Figure 3.2. Average area of habitat growth (black bars) and loss (grey bars) in kilometers for functional groups of grassland species modeled where (A) represents growth and loss within the low carbon emissions scenario, and (B) for the high carbon emissions scenario. Different letters signify significant difference among functional groups after a Tukey-Kramer post-hoc analysis, black letters indicate significant differences in habitat growth and grey letters indicate differences for habitat loss within a functional growth. Warm-season grasses had the highest and most variable growth and loss across both scenarios.

3.5. Discussion

We observed individual species' climatic niche were largely influenced by different

combinations of climate variables. This indicates that although many of these species co-exist

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within similar habitats, individual species-climate relationships are unique. We also quantified future species habitat suitability changes in response to two climate change scenarios, including scenarios of both high and low carbon emission. Across functional groups, total amounts of habitat reduction and gain were not different across the two scenarios, indicating climate change will likely have similar impacts on habitat suitability. Some functional groups had consistently lower amounts of habitat change and may be less of a concern for use in restoration seed mixes, while other functional groups had high variability. Thus, it may be more important to consider individual species' responses to climate change for determining which species to include in seed mixes, particularly for functional groups with high variability in predicted habitat suitability change. Additionally, these results provide a way to optimize seed collections for restoration seed mixes to ensure seed is collected from regions of high suitability regardless of climate change scenario maintaining individual species' on the landscape.

3.5.1. Climate Variables That Influence Species Distributions

For restoring and conserving grassland communities, it may be important to consider a species' climactic niche as individual species may be influenced by different climate variables. For the 26 North American grassland species we modeled, we found individual species were in fact influenced by different climate predictors of seasonal variation in precipitation and temperature (Figure 3.1). The climate predictors retained within our study matched some of those used within Martinson et al. (2011), which also modeled plant species distributions in North American grasslands. By retaining similar climate variables across studies, this may indicate that these variables are in fact important determinants of the climactic niche. This is important as modeling approaches may be skewed if inappropriate climate-relationships are modeled (Williams et al. 2012; Elith & Leathwick 2009). Similarly, some individual species

modeled across our studies did show differences in climate variable importance. For example, Martinson et al. 2011 found the climate factor that was the greatest predictor of *Amorpha canescens'* (Leadplant) distribution was mean annual temperature, whereas we found mean temperature of the warmest quarter to be most important (Figure 3.1). Interestingly, although mean annual temperature was important for many of our species, it was not included in the final model for leadplant. Differences in variable contributions may be expected because while Martinson et al. (2011) used the same nine variables across all 30 of their species, our study evaluated climate variables for individual species. This adds to the ongoing argument of whether variable selection should be completed *a priori* to modeling based on biological assumptions or through correlation analysis to remove highly correlated variables (Ashcroft et al. 2011; Melo-Merino et al. 2020). Based on the differences in variable selection across the multiple studies modeling grassland species distributions, it may be important to identify which variables are consistently retained as they are likely important variables determining the climactic niche. Additionally, identifying common predictors across species could be important for further modeling grassland communities' responses to climate change if using multiple species. Our results show that it may be important to consider individual species' climactic niches when undergoing variable selection analysis. We found that individual species responded to different climate variables and to varying degrees. Some common variables such as mean annual temperature or temperature seasonality were often retained for multiple species and thus should potentially be included in future modeling studies. The varied use of these predictors may also impact how climate change scenarios predict changes in habitat suitability.

3.5.2. Predicted Habitat Suitability Changes

Under future climate scenarios all species were predicted to experience gains and losses in habitat suitability. Given that individual species were modeled and exhibited different climate factors underlying their ecological niche, we expected that functional group responses would vary too. Across both scenarios, late-blooming forbs and legumes were two functional groups that exhibited minimal habitat suitability change in both reduction and expansion in response to climate change scenarios (Figure 3.2). Land managers may potentially use these functional groups as reliable indicators of climate response, rather than by individual species, as these groups are known to have predictable responses to climate change based on the subsequent changes in nutrient availability and productivity (Reich et al. 2001). Previously, Lyon et al. (2019) suggested that forb responses to climate change may be highly conserved. Our results suggest that this may not be the case for all forb species, as habitat change was highly variable particularly for early-blooming forbs. Contrary to Lyon (2019), however, this may be expected as we separated forbs into classes of early and late-blooming rather than together as one. As the seasonal timing of bloom varies for different forb species, the climactic niches these species experience may be different across the growing season as well. Therefore, it may be necessary to tease apart early and late- blooming forb responses to changing climate. Like Lyon et al. (2019), we do consistently observe that legumes had less habitat suitability change, which could suggest some resiliency of this group to changing climatic conditions. These results similarly suggest that some, but not all, functional groups are good indicators of climate response. Both Lyon et al. (2019) and our study had an unequal number of species represented between our functional groups. Within our study, warm-season grasses had high variability in habitat change that was significantly different from some functional groups. Given that only three species composed this

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functional group, this may impact how strong our predictions of functional group habitat suitability may be (Lyon et al. 2019). The variability in predicted habitat suitability changes may indicate that SDM predictions that group individual species into functional groups may be less accurate or informative for restoration seed mix choices.

Functional groups are still an important consideration for biodiversity conservation, and group modeling methods may provide a baseline guide to show how grassland communities may be impacted by climate change. However, community compositions fluctuate and species are not likely to respond similarly under climate change due to differing climatic niches (Guisan & Zimmermann 2000). As such, previous results have shown that modeling individuals over communities may yield more accurate responses to climate change that can be lost at a community-level (Baselga & Araújo 2009; Gogol-Prokurat 2011). Still, model predictions may be improved by accounting for species-interactions that occur within communities, as this may more accurately represent a species' realized niche (Wisz et al. 2013). These data are often hard to collect but may be a tool to consider for further modeling. Where species-interaction data do exist, modeling techniques are advancing to account for interactions that can affect community assemblages (Wisz et al. 2013; Norberg et al. 2019). These analyses could be a potential tool to evaluate community-level responses to climate change in the future (Maguire et al. 2015). Our study suggested functional groups responded differently to climate change scenarios. For legumes and late-blooming forbs that had less habitat change across climate change scenarios, these functional groups may include that species could be used interchangeably within seed mixes. For groups such as asters and warm-season grasses that had high variability in habitat suitability change; however, it may be more useful to make species-specific inclusions for seed-mix purposes as some species may be more sensitive to change than others. Additionally, our

models identify regions within a species' range that are currently suitable and may be used as potential sources for seed mixes. These models also identify parts of the range that may remain suitable under climate change scenarios and could be used to prioritize sampling within regions that will be suitable no matter future climate projections.

Of the functional groups we considered, warm-season grasses had the largest average amount of predicted habitat suitability loss and gain across both scenarios. Within this functional group, *Bouteloua gracilis* stood out as the species with the highest habitat change for both reduction and gain under both scenarios. This species has an extended distribution from the Northern Great Plains into New Mexico and may spread quickly through rhizomes that produce large clumps (Weaver 1954; Lauenroth et al. 1994). Additionally, *B. gracilis* may establish quickly within moist soil, and once established it is largely drought-resistant (Weaver 1954; Lauenroth et al. 1994). With the rapid establishment capacity and the relative resistance of warm-season grasses such as *B. gracilis* to climate events, our data suggest this species could expand its range into new regions as conditions become more favorable. However, even though we might expect this species to be tolerant to climate change, the unpredictability in climate events associated with climate change may be extreme enough to reduce overall species productivity and survival across the species' range (Polley et al. 2013). These models only consider climate, and do not include other considerations of biotic or other abiotic relationships that may also impact species range changes. For the Northern Great Plains region, *B.gracilis* is predicted to maintain high habitat suitability, with suitability potential growing within the core range of the central region (Figure 3.3); however, suitability potential decreases at this species' northeastern range limits across both climate change scenarios (Figure 3.3BC). Importantly, our individual species modeling only represent possible predictions that do not include data on how

species-interactions or boundaries to dispersal may inhibit actual species growth into new regions. By modeling individual habitat suitability, it may provide easily identifiable regions to target for optimizing seed sourcing collections.

Within grassland restorations, building natural communities often requires species-specific decisions to use in seed collections. SDMs provide critical guidance regarding where seed may be sourced to be both predictive and reactive to climate change (Havens et al. 2015; Potter & Hargrove 2012). These choices may be improved by modeling individual species responses to climate change to identify suitable habitats to collect seed from under contemporary climates. Further, by modeling current and future climatic niches in response to climate change, SDMs provide a platform to test hypotheses of predicted species responses to changing CO_2 conditions that can be empirically evaluated in the lab- or field-based experiments. For example, increased CO_2 levels may advance C_3 cool-season grass establishment and production earlier into the spring (Polley et al. 2013). Within grasslands, *Poa pratensis* (Kentucky bluegrass) is a particularly aggressive cool-season grass that readily invades and outcompetes native grass and forb species (DeKeyser et al. 2015). If increased CO_2 facilitates establishment of earlier cool-season grasses there is substantial concern that exotic grasses like *P. pratensis* will continue to outcompete and exclude native plant species from establishing already threatened native grasslands (Polley et al. 2013; Palit et al. 2021). However, the effect of experimentally increased CO_2- levels across various perennial grass and forb species is not consistent, with some species showing no effect of CO_2 on species productivity (Newingham et al. 2014). Quantifying species individual response to contemporary conditions and potential future conditions under multiple CO_2 projections is needed to evaluate the potential long-term impacts of climate change on

grassland species. These results inform future seed sourcing choices to mitigate effects of climate change on already imperiled grassland systems.

Our results show that modeling individual species may be the necessary first step to understanding differing climactic niches of grassland communities. Once we understand how individual species may respond to climate change, we may then use this information to understand how communities may respond. Community-assembly responses may be evaluated where habitats are consistently suitable across multiple-species to stream-line seed collection efforts.
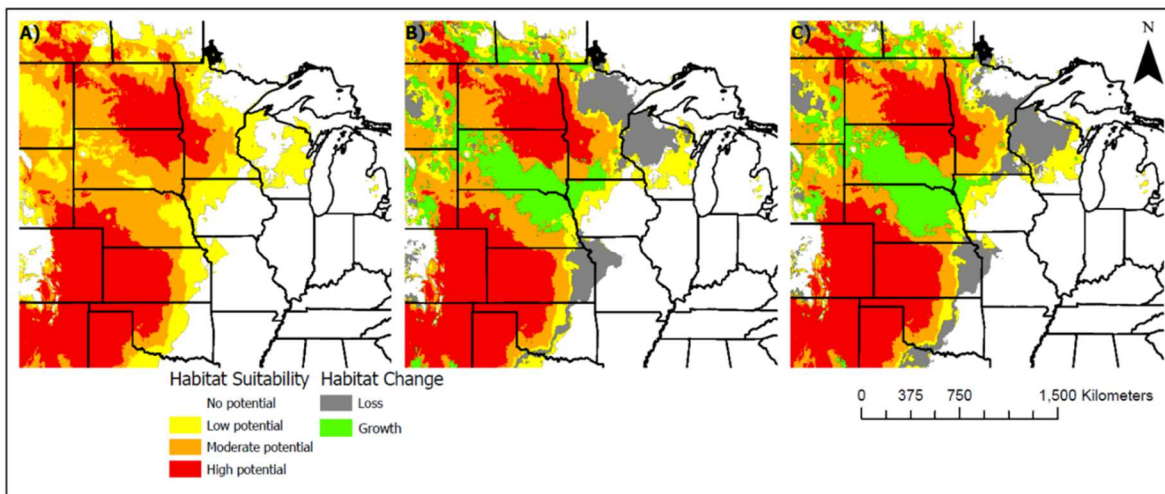

Figure 3.3. Habitat suitability models of *Bouteloua gracilis* (Blue grama) under (A) near current climate conditions, and future climate change scenarios of (B) low carbon emissions (SSP 126) and (C) high carbon emissions (SSP 370). Binned categories are used for easier visualization of habitat suitability where No potential = 0 – 0.2, Low potential = 0.2 – 0.4, Moderate potential = 0.4 – 0.6, and High potential = 0.6 – 1. Habitat change is indicated as Loss where future – current distribution probabilities < 0 and Growth where future – current > 0. No change in suitability (future – current = 0) is indicated by original habitat suitability bin.

3.6. Conclusions

In this study, we identified which climate variables influenced 26 common grassland plant species within the Great Plains region. Although these species are commonly found together in grassland communities and thus experience similar climates, we found that not all

climate predictors contributed to species' ecological niche in the same way. For restoration and establishing seed-mixes it is necessary to evaluate which species are included in a seed mix to ensure biodiversity is maintained regardless of how future climate may change. Using different climate variables, we were able to reliably model and predict individual species' contemporary and future habitat suitability under climate change scenarios. These models allow restoration practitioners to consider not only which individual species to include in a seed mix, but also help visualize where habitat maintains high suitability under climate change scenarios. By modeling two different climate change scenarios, a low and high carbon emission scenario, these data provide a way to optimize seed collection efforts to ensure seed is collected from high suitability sites regardless of how climate changes. Due to the nature of habitat suitability modeling and SDM techniques, results presented here represent a modeled approach to quantify potential habitat suitability and not actualized suitability (Araújo & Peterson 2012). Future work should incorporate additional factors that may influence distributions such as land cover and biotic interactions to create models that may more accurately discern realized habitat suitability and aid in restoration efforts.

3.7. Future Directions

Results presented for this chapter provide necessary insight into how individual species might respond to climate change and provide information restoration practitioners may use to guide seed collection efforts. A next step in data analysis will be to collectively identify habitat suitability across all species. As demonstrated, it may be more informative to model individual species to capture the most relevant climate variables and more reliably predict habitat suitability than by modeling functional groups. However, producing individual species maps for the purposes of seed collection may not be the most practical as seed collections for restoration

typically focus on multiple species. By combining the raster data of habitat suitability across all species, we should be able to find areas of high suitability overlap that may aid in seed collections for more than one species at a time and identify suitable areas to implement seed mixes for restoration.

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4. RESEARCH SUMMARY AND FUTURE DIRECTIONS

The objectives of this thesis were to assess two key components of ecological restoration success: i) We empirically evaluated the role of seed source diversity on establishment during the initial phases of restoration, and ii) we identified climate factors that influence grassland species presence on the landscape and modeled habitat suitability for those species under a range of scenarios of climate change. Understanding how within species diversity contributes to early plant establishment may aid in guiding seed mix design choices used in restorations (Bucharova et al. 2019). Additionally, by modeling the relationship between climate and species presence on the landscape, we can use our understanding of species' climatic niche to inform both seed sourcing choices within areas that remain highly suitable under current conditions and identify regions where species may be appropriate for restoration under future climate scenarios (Havens et al. 2015).

4.1. Research Summary

We found that during the first year of restoration establishment, diversity was not impacted by the application of single versus multiple seed sources within each species for our restoration seed mix treatments. The majority of species that emerged were non-seeded species, although this varied across the two experimental restoration sites. These results are consistent with other restoration experiments that indicate that early-stage restorations may be dominated by non-native species (Piper et al. 2007; Déri et al. 2011; Martin & Wilsey 2014). Our results suggest that early emergence and diversity following restoration is largely influenced by the variation in land-use history and local seedbanks. For the RSC restoration site, micro-site heterogeneity influenced community composition diversity, and not the influence of seed mix treatment or type within each seed mix treatment plot group. Our results for the ORD site

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similarly showed no influence of seed mix type, however, community composition was similar across all seed mix treatments over time. Suggesting that there was minimal, micro-site variation across the restoration plots to influence community emergence. These communities had similar patterns of species emergence across the growing season, indicating that land-use history is likely playing a determining role in initial plant establishment.

In addition, we modeled habitat suitability for 26 grassland species that are commonly incorporated into restoration seed mixes in the Northern Great Plains (Smith 2010). We observed that species' climatic niches were largely influenced by different climate variables. This indicates that although many of these species co-exist within similar habitats, individual species-climate relationships are unique. For modeling it may be more appropriate to consider individual species first to capture what climate is driving presences across the landscape and then combine individual species outputs as a group to quantify areas of high suitability overlap. Although restoration seed source decisions are typically made to ensure functional diversity persists (Oakley & Knox 2013; Norland et al. 2013), our results may help restoration practitioners evaluate individual species within these groups to identify current areas of high habitat suitability for contemporary seed sourcing. We also quantified future species habitat suitability changes in response to two climate change scenarios, including scenarios of both high and low carbon emission. Total amounts of habitat reduction and gain were not different across the two scenarios, indicating any amount of climate change likely will have an impact in species habitat suitability. If considering functional groups for inclusion in restoration seed mixes, it may be necessary to make careful considerations for which species within groups to include. For example, warm-season grasses as these species showed both high reductions and gains, and modeled habitat suitability may show where habitat change may occur. These results may

provide a way to optimize seed collections for restoration seed mixes to ensure seed is collected from regions of high suitability regardless of climate change scenario to maintain individual species' persistence on the landscape.

4.2. Future Directions

Future work should emphasize long-term assessments across multiple years to quantify the impact of seed mix type to community diversity and restoration success over time. Second year community data was collected from our experimental sites within June and August of 2021 and will be added to our analysis to evaluate community composition changes across seed mix treatment types. Previous studies have shown that the establishment of seed mix species within the first year positively influences establishment through increased seed mix species abundance and richness in subsequent years following restoration (Applestein et al. 2018; Geaumont et al. 2019), thus we expect to see greater seeded species richness and community diversity within both experimental restoration sites in the future. Additionally, it may be beneficial to evaluate what seeded and non-seeded species persist across years to help inform future restoration management plans for long-term restoration success.

For habitat suitability modeling to inform restoration decisions, it may be beneficial to evaluate how different modeling algorithms compare in modeling predictions. MaxEnt modeling is most often used in creating habitat suitability modeling over other SDM approaches for its high predictive accuracy and ability to work with presence-only data (Bradie & Leung 2017; Melo-Merino et al. 2020). However, alternative modeling approaches may be used in conjunction with MaxEnt to create comprehensive ensemble comparisons of habitat suitability (Norberg et al. 2019). Previous results have shown MaxEnt assessed individually may perform better than other algorithms and can be evaluated on its own or at least should be evaluated

alongside an ensemble (Grimmett et al. 2020). Limits to MaxEnt are that modeling requires presence-only data and modeling methods may be inadequate if the data has sampling bias or does not capture the full species range (Pearson & Dawson 2003; Phillips et al. 2009). Additionally, modeling for all algorithms is only as accurate as the data that is used to model species-climate relationships. Thus, careful considerations are needed for environmental variables used for model prediction. If too many or inappropriate explanatory variables are used, then modeled ranges may be skewed or inaccurate (Williams et al. 2012; Elith & Leathwick 2009). An ensemble approach may be used to identify which common variables are retained across variables and may be used to reduce model uncertainty (Grimmett et al. 2020; Williams et al. 2012). Further benefits to ensemble modeling with multiple algorithm approaches are that it may create higher predictive accuracy in some instances, and create maps that are more conservative in suitability estimates (Rosner-Katz et al. 2020; Grimmett et al. 2020). Ensemble modeling could thus be an approach to increase modeling sensitivity and potentially may yield results that more accurately reflect greater resolutions of habitat suitability for identifying areas of low concern (consistently high suitability) or high concern (areas that are low in suitability). As MaxEnt models produced in this thesis had high predictive accuracy, a future direction will be to overlay suitability predictions across all modeled species to identify regions with suitability overlap. This may aid restoration practitioners in both making seed sourcing decisions for multiple species within a single area and identifying regions to implement restorations in the future.

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APPENDIX A. CHAPTER TWO SUPPLEMENTAL MATERIAL

Table A1. Individual guides with links used to calculate the species-specific number of seeds found per gram for each species used in restoration seed treatments.

Species Scientific Name	Guide Source
Amorpha canescens	Prairie Moon
Anemone cylindrica	Native Seed Production Manual
Artemisia frigida	L&H Seed
Bouteloua curtipendula	Native Seed Production Manual
Bouteloua gracilis	Prairie Moon
Dalea purpurea	Native Seed Production Manual
Echinacea angustifolia	Prairie Moon
Geum triflorum	Prairie Moon
Helianthus maximiliani	Prairie Moon
Helianthus pauciflorus	Prairie Moon
Hesperostipa comata	USDA Plant Database
Liatris punctata	Prairie Moon
Pediomelum argophyllum	Shirley 1994. Restoring the Tallgrass Prairie: An Illustrated Manual for Iowa and the Upper Midwest.
Potentilla arguta	Prairie Moon
Ratibida columnifera	Prairie Moon
Schizachriym scoparium	Native Seed Production Manual
Solidago rigida	Native Seed Production Manual

Note: Resource links for guides: https://www.prairiemoon.com/, https://tallgrassprairiecenter.org/sites/default/files/native_seed_production_manual.pdf, http://www.lhseeds.com/artemisia-frigida-fringed-sagebrush, https://plants.usda.gov/plantguide/pdf/pg_heco26.pdf

Table A2. Pairwise geographic distances in kilometers between individual seed collection sites from remnant prairies sampled throughout northwestern Minnesota. Pairwise distances ranged from 3.11 km to a maximum of 215.13 km.

	GRP	AGD	TWI	FMB	ZIM	BIC	FLI	BLU	OLS	BLA	HAN	SEV	POM
AGD	22.84	-	-	-	-	-	-	-	-	-	-	-	-
TWI	59.89	37.09	-	-	-	-	-	-	-	-	-	-	-
FMB	52.88	30.40	8.62	-	-	-	-	-	-	-	-	-	-
ZIM	68.45	46.29	17.86	25.37	-	-	-	-	-	-	-	-	-
BIC	74.88	52.20	15.43	22.10	22.36	-	-	-	-	-	-	-	-
FLI	73.66	50.89	13.81	21.27	19.25	3.11	-	-	-	-	-	-	-
BLU	96.73	74.05	37.09	43.92	37.37	21.86	23.31	-	-	-	-	-	-
OLS	94.64	71.96	36.44	44.93	27.39	25.98	24.95	19.37	-	-	-	-	-
BLA	114.37	91.65	55.66	63.97	46.88	43.34	43.10	26.97	19.74	-	-	-	-
HAN	150.04	127.23	90.52	98.40	83.16	76.63	77.13	55.98	55.85	36.32	-	-	-
SEV	183.54	161.70	128.18	136.75	115.42	117.27	116.70	100.31	91.93	74.18	51.64	-	-
POM	150.51	127.68	90.67	98.23	84.86	76.19	77.02	54.81	57.49	38.75	8.17	58.52	-
STA	215.13	193.04	158.63	167.11	146.77	146.80	146.53	128.29	122.19	103.47	74.50	32.36	79.33

Table A3. Geographic distances in km between individual seed collection sites to established restoration plots at the RSC site within northwestern Minnesota. Distances ranged from 2.22 km to 129.27 km.

Site Code	Distance (km)
GRP	94.82
AGD	72.10
TWI	35.08
FMB	42.06
ZIM	35.15
BIC	19.96
FLI	21.28
BLU	2.22
OLS	17.96
BLA	27.25
HAN	57.37
SEV	100.99
POM	56.43
STA	129.27

Table A4. Pairwise geographic distances in kilometers between individual seed collection sites from remnant prairies sampled throughout the Missouri Coteau region. Pairwise distances ranged from 2.38 km to a maximum of 311.56 km.

	MYR	GRO	COR	NBM	KRU	MUN	KOS	LSB	ORD	TEN	EUR	RYM	GDY	ARF	JNK	MIL
GRO	11.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
COR	27.66	16.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NBM	24.80	33.56	46.52	-	-	-	-	-	-	-	-	-	-	-	-	-
KRU	16.98	18.42	27.62	19.87	-	-	-	-	-	-	-	-	-	-	-	-
MUN	27.47	28.09	33.68	22.02	10.56	-	-	-	-	-	-	-	-	-	-	-
KOS	30.54	28.81	31.28	28.13	13.87	6.14	-	-	-	-	-	-	-	-	-	-
LSB	66.62	59.90	50.96	66.86	52.07	45.09	39.27	-	-	-	-	-	-	-	-	-
ORD	95.95	92.51	87.37	86.65	79.18	69.24	65.41	39.35	-	-	-	-	-	-	-	-
TEN	89.26	88.22	86.60	75.45	72.32	61.79	59.52	46.50	19.95	-	-	-	-	-	-	-
EUR	93.81	95.34	97.05	75.31	77.67	67.35	66.95	64.46	40.31	20.96	-	-	-	-	-	-
RYM	143.77	142.45	139.47	128.96	126.84	116.31	113.91	92.81	53.46	54.52	56.29	-	-	-	-	-
GDY	180.80	180.02	177.46	164.59	163.95	153.39	151.33	130.70	91.38	91.81	89.73	38.05	-	-	-	-
ARF	192.58	190.04	185.01	179.39	175.61	165.24	162.19	135.25	97.75	104.06	107.58	51.29	30.24	-	-	-
JNK	194.96	192.40	187.34	181.77	177.99	167.62	164.57	137.54	100.10	106.44	109.91	53.62	31.51	2.38	-	-
MIL	242.63	239.37	233.14	230.44	225.73	215.50	212.11	182.48	146.89	154.99	159.14	102.85	75.43	51.56	49.23	-
NIE	311.56	306.93	298.69	301.84	294.97	285.16	281.13	247.81	215.93	226.83	233.32	177.29	151.75	126.58	124.38	76.32

Table A5. Geographic distances in km between individual seed collection sites to established restoration plots at the ORD site within the Missouri Coteau. Distances ranged from 3.54 km to 214.04 km.

Site Code	Distance (km)
MYR	97.61
GRO	93.80
COR	88.09
NBM	89.04
KRU	80.94
MUN	71.15
KOS	67.12
LSB	39.13
ORD	3.54
TEN	23.48
EUR	43.77
RYM	53.94
GDY	91.65
ARF	96.91
JNK	99.25
MIL	145.59
NIE	214.04

Table A6. Cover-class method used to quantify coverage estimates for individual species, litter cover, and bare ground soil coverage modified from Daubenmire (1959). Estimates were taken for all quadrats sampled and averaged to obtain a plot-replicate level estimates of coverage.

Code	Estimated cover range
0	0-4%
5	5-9%
10	10-19%
20	20-29%
30	30-39%
40	40-49%
50	50-59%
60	60-69%
70	70-79%
80	80-89%
90	90-94%
95	95-99%
100	100%

Table A7. RSC pairwise comparisons evaluating differences in community composition by seed treatment. Data is subset by month of data collection to account for significant PERMANOVA interaction between seed treatment and month on community composition. Within this analysis the multiple-source mix communities were significantly different from all single-source mixes. except for seed source E in August and September

June					July				
Pairs	SS	F	R2	p	Pairs	SS	F	R2	p
A vs B	0.62	4.71	0.54	0.10	A vs B	0.72	10.61	0.73	0.10
A vs C	0.70	4.16	0.51	0.10	A vs C	0.75	5.28	0.57	0.10
A vs D	0.71	3.49	0.47	0.10	A vs D	0.41	2.22	0.36	0.10
A vs E	0.69	11.82	0.75	0.10	A vs E	0.77	11.55	0.74	0.10
A vs ABCDE	1.25	10.48	0.64	*0.02*	A vs ABCDE	1.27	13.42	0.69	*0.02*
B vs C	0.73	3.26	0.45	0.10	B vs C	0.73	4.12	0.51	0.10
B vs D	0.68	2.63	0.40	0.20	B vs D	0.57	2.63	0.40	0.10
B vs E	0.57	5.02	0.56	0.10	B vs E	0.38	3.75	0.48	0.10
C vs D	0.14	0.46	0.10	0.60	C vs D	0.21	0.73	0.15	0.20
C vs E	0.92	6.16	0.61	0.10	C vs E	0.82	4.67	0.54	0.10
D vs E	0.91	4.92	0.55	0.10	D vs E	0.65	2.99	0.43	0.10
ABCDE vs B	1.12	7.18	0.54	*0.02*	ABCDE vs B	1.09	9.27	0.61	*0.02*
ABCDE vs C	1.13	6.26	0.51	*0.02*	ABCDE vs C	1.12	6.66	0.53	*0.02*
ABCDE vs D	1.11	5.43	0.47	*0.02*	ABCDE vs D	0.93	4.77	0.44	*0.02*
ABCDE vs E	0.82	7.62	0.56	*0.02*	ABCDE vs E	0.60	5.13	0.46	*0.02*

August					September				
Pairs	SS	F	R2	p	Pairs	SS	F	R2	p
A vs B	0.48	7.87	0.66	0.10	A vs B	0.32	3.41	0.46	0.10
A vs C	0.51	4.02	0.50	0.10	A vs C	0.57	3.25	0.45	0.10
A vs D	0.42	1.82	0.31	0.10	A vs D	0.33	1.60	0.29	0.20
A vs E	0.30	3.66	0.48	0.10	A vs E	0.61	5.59	0.58	0.10
A vs ABCDE	0.77	8.68	0.59	*0.02*	A vs ABCDE	0.73	5.13	0.46	*0.02*
B vs C	0.64	4.31	0.52	0.10	B vs C	0.69	3.67	0.48	0.10
B vs D	0.43	1.72	0.30	0.10	B vs D	0.66	2.97	0.43	0.20
B vs E	0.56	5.47	0.58	0.10	B vs E	0.45	3.71	0.48	0.10
C vs D	0.14	0.44	0.10	0.90	C vs D	0.25	0.84	0.17	0.20
C vs E	0.38	2.27	0.36	0.30	C vs E	0.62	3.05	0.43	0.10
D vs E	0.26	0.95	0.19	0.40	D vs E	0.72	3.05	0.43	0.10
ABCDE vs B	0.84	8.13	0.58	*0.02*	ABCDE vs B	0.87	5.80	0.49	*0.02*
ABCDE vs C	0.66	4.51	0.43	*0.02*	ABCDE vs C	0.80	3.90	0.39	*0.02*
ABCDE vs D	0.44	2.04	0.25	*0.04*	ABCDE vs D	0.77	3.42	0.36	*0.02*
ABCDE vs E	0.23	2.00	0.25	0.07	ABCDE vs E	0.22	1.35	0.18	0.31

Table A8. ORD pairwise comparisons on community diversity differences between month of data collection. Community compositions were significantly different in June compared to August and September.

Pairs	SS	F	R2	p
June vs July	0.16	1.11	0.10	0.36
June vs August	0.27	2.27	0.19	*0.05*
June vs September	0.30	2.51	0.20	*0.02*
July vs August	0.24	1.96	0.16	0.11
July vs September	0.20	1.57	0.14	0.16
August vs September	0.08	0.80	0.07	0.58

Table A9. Species collection information for northwestern MN seed mixes sorted by species, the single-source seed mix individual species were used in (A,B,C,D,E), the location code species were sourced from (code is labeled by US state of collection, region name, and a unique three letter combination identifying site), and the location of site by latitude and longitude.

Species Scientific Name	Mix	Location	Latitude	Longitude
Amoprha canescens	A	MN-ABR-AGD	47.51152	-96.29388
Amoprha canescens	B	MN-ABR-BIC	47.0507	-96.42639
Amoprha canescens	C	MN-ABR-BLU	46.85638	-96.47015
Amoprha canescens	D	MN-ABR-BLA	46.68888	-96.21477
Amoprha canescens	E	MN-ABR-POM	46.36548	-96.40331
Anemone cylindrica	A	MN-ABR-AGD	47.51152	-96.29388
Anemone cylindrica	B	MN-ABR-BIC	47.0507	-96.42639
Anemone cylindrica	C	MN-ABR-BLU	46.85638	-96.47015
Anemone cylindrica	D	MN-ABR-BLA	46.68888	-96.21477
Anemone cylindrica	E	MN-ABR-SEV	46.10707	-95.74233
Artemisia frigida	A	MN-ABR-AGD	47.51152	-96.29388
Artemisia frigida	B	MN-ABR-BIC	47.0507	-96.42639
Artemisia frigida	C	MN-ABR-BLU	46.85638	-96.47015
Artemisia frigida	D	MN-ABR-FMB	47.24906	-96.4069
Artemisia frigida	E	MN-ABR-SEV	46.10707	-95.74233
Bouteloua curtipendula	A	MN-ABR-AGD	47.51152	-96.29388
Bouteloua curtipendula	B	MN-ABR-BIC	47.0507	-96.42639
Bouteloua curtipendula	C	MN-ABR-BLU	46.85638	-96.47015
Bouteloua curtipendula	D	MN-ABR-HAN	46.3671	-96.29711
Bouteloua curtipendula	E	MN-ABR-POM	46.36548	-96.40331
Dalea purpurea	A	MN-ABR-AGD	47.51152	-96.29388
Dalea purpurea	B	MN-ABR-BIC	47.0507	-96.42639
Dalea purpurea	C	MN-ABR-BLU	46.85638	-96.47015
Dalea purpurea	D	MN-ABR-BLA	46.68888	-96.21477
Dalea purpurea	E	MN-ABR-POM	46.36548	-96.40331
Echinacea angustifolia	A	MN-ABR-TWI	47.18041	-96.35409
Echinacea angustifolia	B	MN-ABR-BIC	47.0507	-96.42639
Echinacea angustifolia	C	MN-ABR-BLU	46.85638	-96.47015
Echinacea angustifolia	D	MN-ABR-OLS	46.866415	-96.21658
Echinacea angustifolia	E	MN-ABR-STA	45.815993	-95.748746
Geum triflorum	A	MN-ABR-GRP	47.716661	-96.278738
Geum triflorum	B	MN-ABR-FMB	47.24906	-96.4069
Geum triflorum	C	MN-ABR-BLU	46.85638	-96.47015

Table A9. Species collection information for northwestern MN seed mixes sorted by species, the single-source seed mix individual species were used in (A,B,C,D,E), the location code species were sourced from (code is labeled by US state of collection, region name, and a unique three letter combination identifying site), and the location of site by latitude and longitude (continued).

Species Scientific Name	Mix	Location	Latitude	Longitude
Geum triflorum	D	MN-ABR-BLA	46.68888	-96.21477
Geum triflorum	E	MN-ABR-SEV	46.10707	-95.74233
Helianthus maximiliani	A	MN-ABR-TWI	47.18041	-96.35409
Helianthus maximiliani	B	MN-ABR-ZIM	47.10778	-96.14406
Helianthus maximiliani	C	MN-ABR-BLU	46.85638	-96.47015
Helianthus maximiliani	D	MN-ABR-BLA	46.68888	-96.21477
Helianthus maximiliani	E	MN-ABR-STA	45.815993	-95.748746
Helianthus pauciflorus	A	MN-ABR-TWI	47.18041	-96.35409
Helianthus pauciflorus	B	MN-ABR-ZIM	47.10778	-96.14406
Helianthus pauciflorus	C	MN-ABR-BLU	46.85638	-96.47015
Helianthus pauciflorus	D	MN-ABR-BLA	46.68888	-96.21477
Helianthus pauciflorus	E	MN-ABR-STA	45.815993	-95.748746
Liatris punctata	A	MN-ABR-AGD	47.51152	-96.29388
Liatris punctata	B	MN-ABR-BIC	47.0507	-96.42639
Liatris punctata	C	MN-ABR-BLU	46.85638	-96.47015
Liatris punctata	D	MN-ABR-BLA	46.68888	-96.21477
Liatris punctata	E	MN-ABR-SEV	46.10707	-95.74233
Pediomelum argophyllum	A	MN-ABR-TWI	47.18041	-96.35409
Pediomelum argophyllum	B	MN-ABR-ZIM	47.10778	-96.14406
Pediomelum argophyllum	C	MN-ABR-BLU	46.85638	-96.47015
Pediomelum argophyllum	D	MN-ABR-OLS	46.866415	-96.21658
Pediomelum argophyllum	E	MN-ABR-STA	45.815993	-95.748746
Potentilla (Drymocallis) arguta	A	MN-ABR-POM	46.36548	-96.40331
Potentilla (Drymocallis) arguta	B	MN-ABR-BIC	47.0507	-96.42639
Potentilla (Drymocallis) arguta	C	MN-ABR-BLU	46.85638	-96.47015
Potentilla (Drymocallis) arguta	D	MN-ABR-BLA	46.68888	-96.21477
Potentilla (Drymocallis) arguta	E	MN-ABR-STA	45.815993	-95.748746
Schizachriym scoparium	A	MN-ABR-TWI	47.18041	-96.35409
Schizachriym scoparium	B	MN-ABR-BIC	47.0507	-96.42639
Schizachriym scoparium	C	MN-ABR-BLU	46.85638	-96.47015
Schizachriym scoparium	D	MN-ABR-BLA	46.68888	-96.21477
Schizachriym scoparium	E	MN-ABR-SEV	46.10707	-95.74233
Solidago rigida	A	MN-ABR-FMB	47.24906	-96.4069
Solidago rigida	B	MN-ABR-ZIM	47.10778	-96.14406
Solidago rigida	C	MN-ABR-BLU	46.85638	-96.47015
Solidago rigida	D	MN-ABR-BIC	47.0507	-96.42639
Solidago rigida	E	MN-ABR-STA	45.815993	-95.748746

Table A10. Species collection information for Missouri Coteau seed mixes sorted by species, single-source seed mix individual species were used in (A,B,C,D,E), the location code species were sourced from (code is labeled by US state of collection, region name, and a unique three letter combination identifying site), and the location of site by latitude and longitude.

Species Scientific Name	Mix	Location	Latitude	Longitude
Amoprha canescens	A	ND-MOCO-NBM	46.452496	-99.492513
Amoprha canescens	B	ND-MOCO-KRU	46.42352	-99.23698
Amoprha canescens	C	SD-MOCO-ORD	45.716309	-99.127932
Amoprha canescens	D	SD-MOCO-GDY	44.97742	-99.63688
Amoprha canescens	E	SD-MOCO-JNK	44.82523	-99.29988
Bouteloua curtipendula	A	ND-MOCO-COR	46.483285	-98.887434
Bouteloua curtipendula	B	ND-MOCO-NBM	46.452496	-99.492513
Bouteloua curtipendula	C	SD-MOCO-ORD	45.716309	-99.127932
Bouteloua curtipendula	D	SD-MOCO-RYM	45.294307	-99.455084
Bouteloua curtipendula	E	SD-MOCO-MIL	44.396799	-99.145596
Bouteloua gracilis	A	ND-MOCO-NBM	46.452496	-99.492513
Bouteloua gracilis	B	ND-MOCO-KRU	46.42352	-99.23698
Bouteloua gracilis	C	SD-MOCO-ORD	45.716309	-99.127932
Bouteloua gracilis	D	SD-MOCO-GDY	44.97742	-99.63688
Bouteloua gracilis	E	SD-MOCO-JNK	44.82523	-99.29988
Dalea purpurea	A	ND-MOCO-NBM	46.452496	-99.492513
Dalea purpurea	B	ND-MOCO-KOS	46.301838	-99.19845
Dalea purpurea	C	SD-MOCO-ORD	45.716309	-99.127932
Dalea purpurea	D	SD-MOCO-TEN	45.780217	-99.367829
Dalea purpurea	E	SD-MOCO-JNK	44.82523	-99.29988
Echinacea angustifolia	A	ND-MOCO-GRO	46.54656	-99.07637
Echinacea angustifolia	B	ND-MOCO-LSB	46.025487	-98.88189
Echinacea angustifolia	C	SD-MOCO-ORD	45.716309	-99.127932
Echinacea angustifolia	D	SD-MOCO-GDY	44.97742	-99.63688
Echinacea angustifolia	E	SD-MOCO-MIL	44.396799	-99.145596
Geum triflorum	A	ND-MOCO-MYR	46.575713	-99.222739
Geum triflorum	B	ND-MOCO-KRU	46.42352	-99.23698
Geum triflorum	C	ND-MOCO-GRO	46.54656	-99.07637
Geum triflorum	D	ND-MOCO-COR	46.483285	-98.887434
Geum triflorum	E	ND-MOCO-MUN	46.3308	-99.26634
Helianthus maximiliani	A	ND-MOCO-NBM	46.452496	-99.492513
Helianthus maximiliani	B	ND-MOCO-KRU	46.42352	-99.23698
Helianthus maximiliani	C	SD-MOCO-ORD	45.716309	-99.127932
Helianthus maximiliani	D	ND-MOCO-COR	46.483285	-98.887434
Helianthus maximiliani	E	SD-MOCO-MIL	44.396799	-99.145596
Helianthus pauciflorus	A	ND-MOCO-GRO	46.54656	-99.07637
Helianthus pauciflorus	B	ND-MOCO-LSB	46.025487	-98.88189
Helianthus pauciflorus	C	SD-MOCO-ORD	45.716309	-99.127932
Helianthus pauciflorus	D	ND-MOCO-COR	46.483285	-98.887434
Helianthus pauciflorus	E	SD-MOCO-MIL	44.396799	-99.145596
Hesperostipa comata	A	ND-MOCO-KRU	46.42352	-99.23698
Hesperostipa comata	B	ND-MOCO-KOS	46.301838	-99.19845
Hesperostipa comata	C	SD-MOCO-ORD	45.716309	-99.127932
Hesperostipa comata	D	SD-MOCO-GDY	44.97742	-99.63688
Hesperostipa comata	E	SD-MOCO-JNK	44.82523	-99.29988
Liatris punctata	A	ND-MOCO-NBM	46.452496	-99.492513
Liatris punctata	B	ND-MOCO-KRU	46.42352	-99.23698
Liatris punctata	C	SD-MOCO-ORD	45.716309	-99.127932

Table A10. Species collection information for Missouri Coteau seed mixes sorted by species, single-source seed mix individual species were used in (A,B,C,D,E), the location code species were sourced from (code is labeled by US state of collection, region name, and a unique three letter combination identifying site), and the location of site by latitude and longitude (continued).

Species Scientific Name	Mix	Location	Latitude	Longitude
Liatris punctata	D	ND-MOCO-COR	46.483285	-98.887434
Liatris punctata	E	SD-MOCO-MIL	44.396799	-99.145596
Pediomelum argophyllum	A	ND-MOCO-NBM	46.452496	-99.492513
Pediomelum argophyllum	B	ND-MOCO-KRU	46.42352	-99.23698
Pediomelum argophyllum	C	SD-MOCO-ORD	45.716309	-99.127932
Pediomelum argophyllum	D	SD-MOCO-GDY	44.97742	-99.63688
Pediomelum argophyllum	E	SD-MOCO-MIL	44.396799	-99.145596
Potentilla (Drymocallis) arguta	A	ND-MOCO-NBM	46.452496	-99.492513
Potentilla (Drymocallis) arguta	B	SD-MOCO-JNK	44.82523	-99.29988
Potentilla (Drymocallis) arguta	C	SD-MOCO-ORD	45.716309	-99.127932
Potentilla (Drymocallis) arguta	D	SD-MOCO-GDY	44.97742	-99.63688
Potentilla (Drymocallis) arguta	E	SD-MOCO-MIL	44.396799	-99.145596
Ratibida columnifera	A	ND-MOCO-NBM	46.452496	-99.492513
Ratibida columnifera	B	SD-MOCO-JNK	44.82523	-99.29988
Ratibida columnifera	C	SD-MOCO-ORD	45.716309	-99.127932
Ratibida columnifera	D	SD-MOCO-MIL	44.396799	-99.145596
Ratibida columnifera	E	SD-MOCO-NIE	43.804709	-98.664164
Solidago rigida	A	ND-MOCO-NBM	46.452496	-99.492513
Solidago rigida	B	ND-MOCO-KRU	46.42352	-99.23698
Solidago rigida	C	SD-MOCO-ORD	45.716309	-99.127932
Solidago rigida	D	ND-MOCO-COR	46.483285	-98.887434
Solidago rigida	E	SD-MOCO-MIL	44.396799	-99.145596

APPENDIX B. CHAPTER THREE SUPPLEMENTAL INFORMATION

Habitat suitability models for individual species during (A) contemporary time 1970-2000, (B) Under low emission carbon scenario in the near future 2021-2040, and (C) Under high carbon emission scenario in the near future 2021-2040. Habitat suitability ranges from binary No suitability (0-0.2), Low suitability (0.2-0.4), Moderate suitability (0.4-0.6), and High suitability (0.6-1). Habitat change is categorized as Loss (where change in suitability is negative; shown in dark grey) and Growth (where change in suitability is positive; shown as green). Areas where suitability does not change is shown in original habitat suitability colors. Figures show the distribution of individual species within the Northern Great Plains region (labeled as "Region Map") and for the species' entire range (labeled as "Range Map").
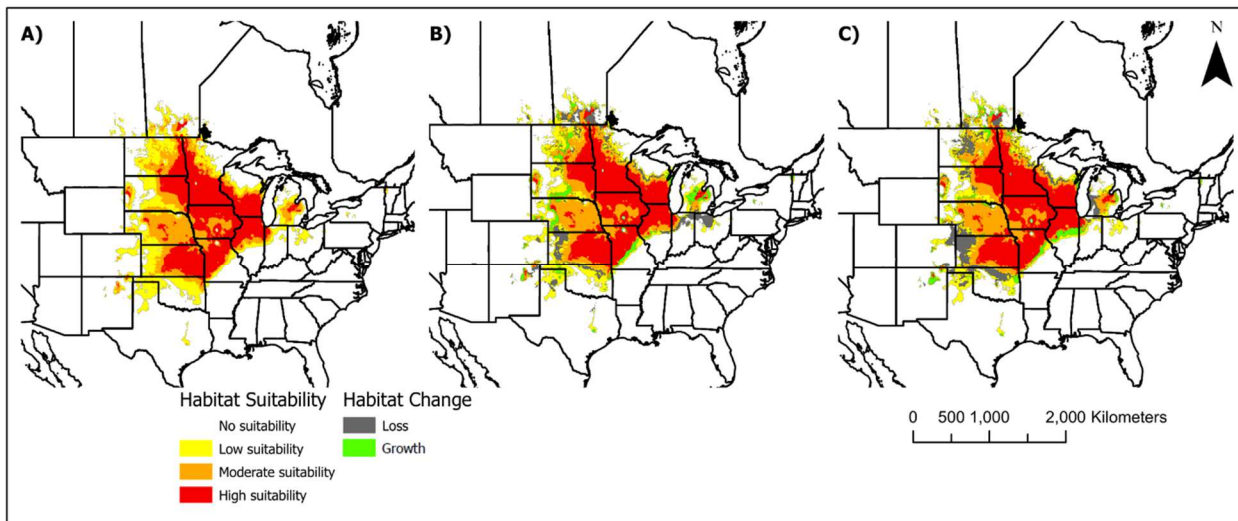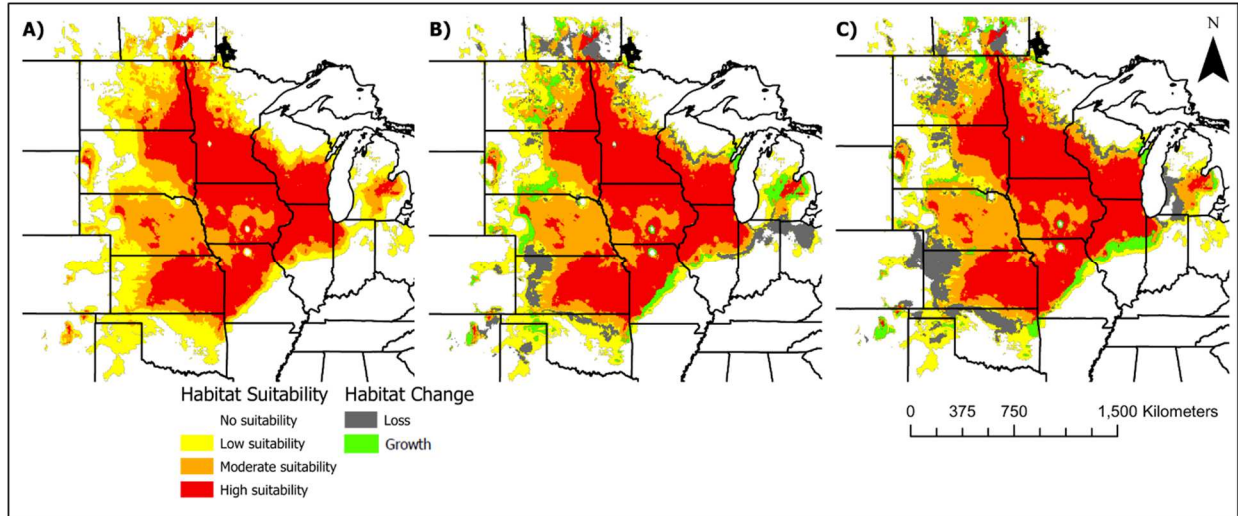
Figure B1. *Amorpha canescens* (Leadplant) – Legume Region Map; *Amorpha canescens* (Leadplant) – Legume Range Map.
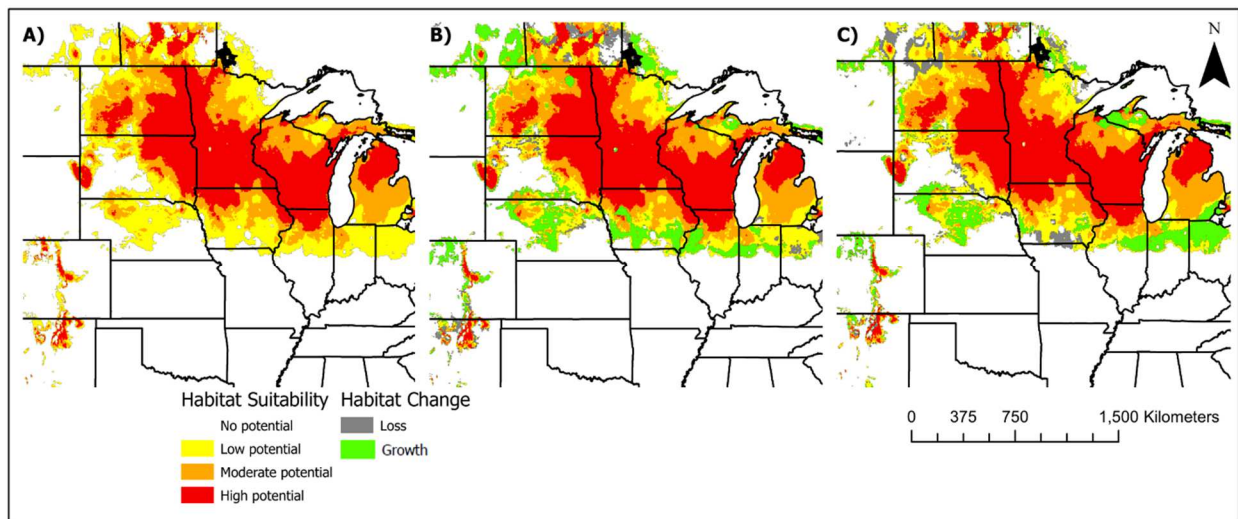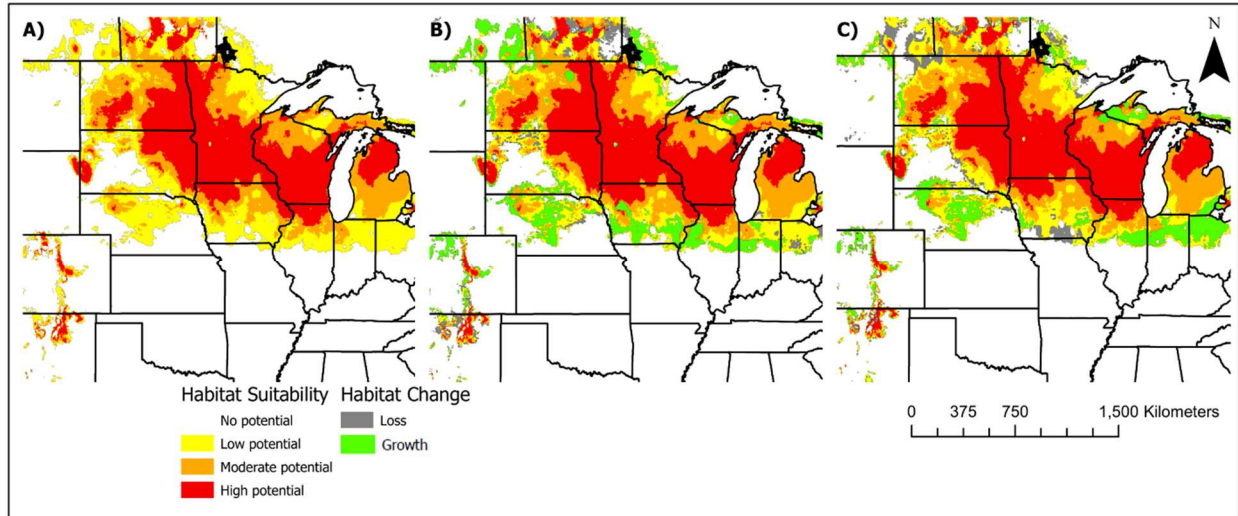
Figure B2. *Anemone cylindrica* (Tall Thimbleweed) – EB-Forb Region Map; *Anemone cylindrica* (Tall Thimbleweed) – EB-Forb Range Map.
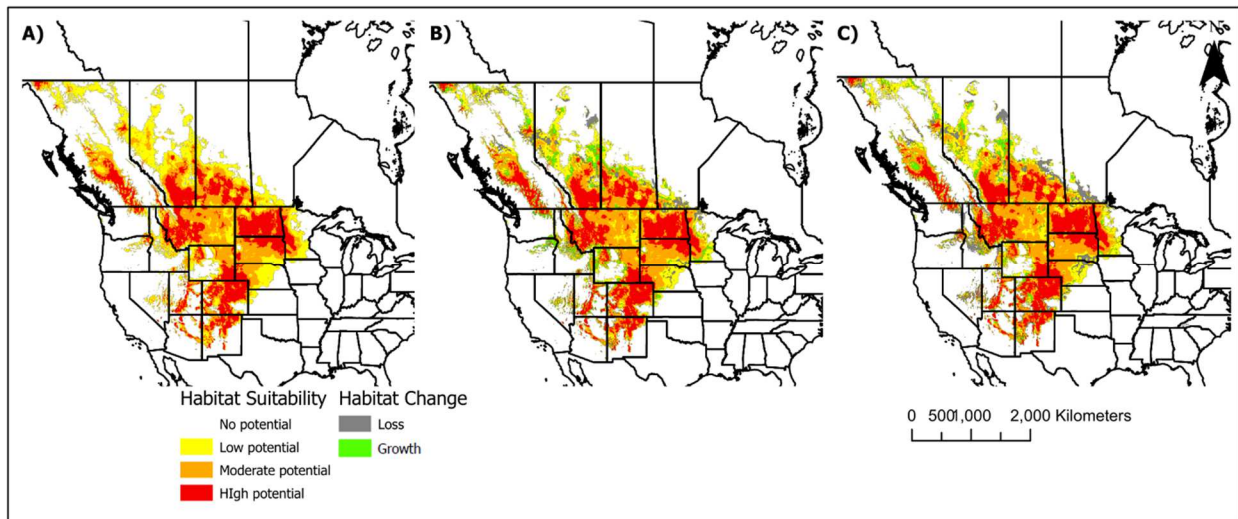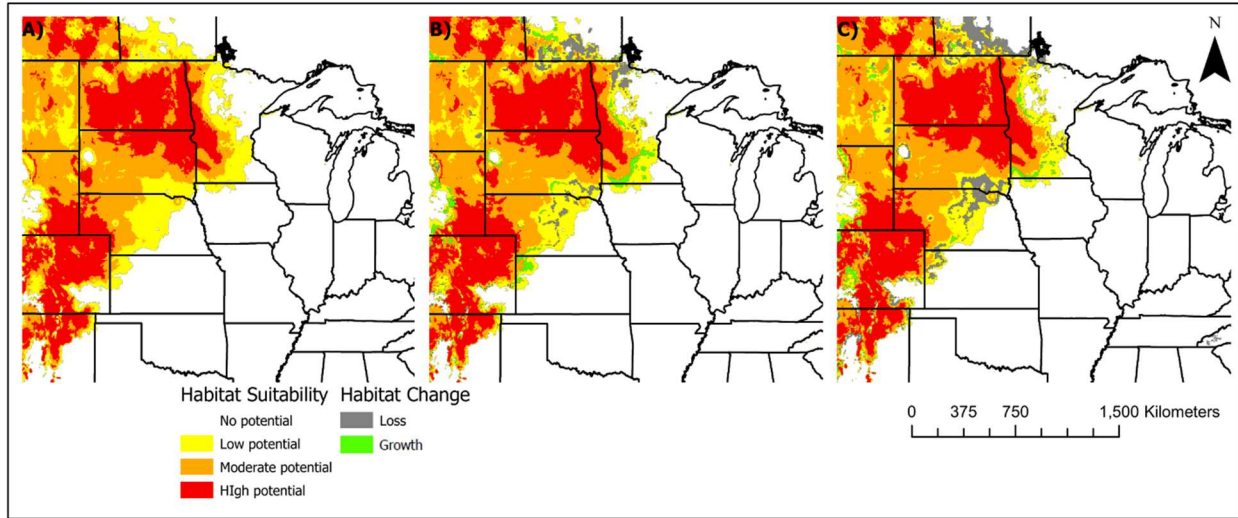
Figure B3. *Artemisia frigida* (Fringed Sage) – Aster Region Map; *Artemisia frigida* (Fringed Sage) – Aster Range Map.
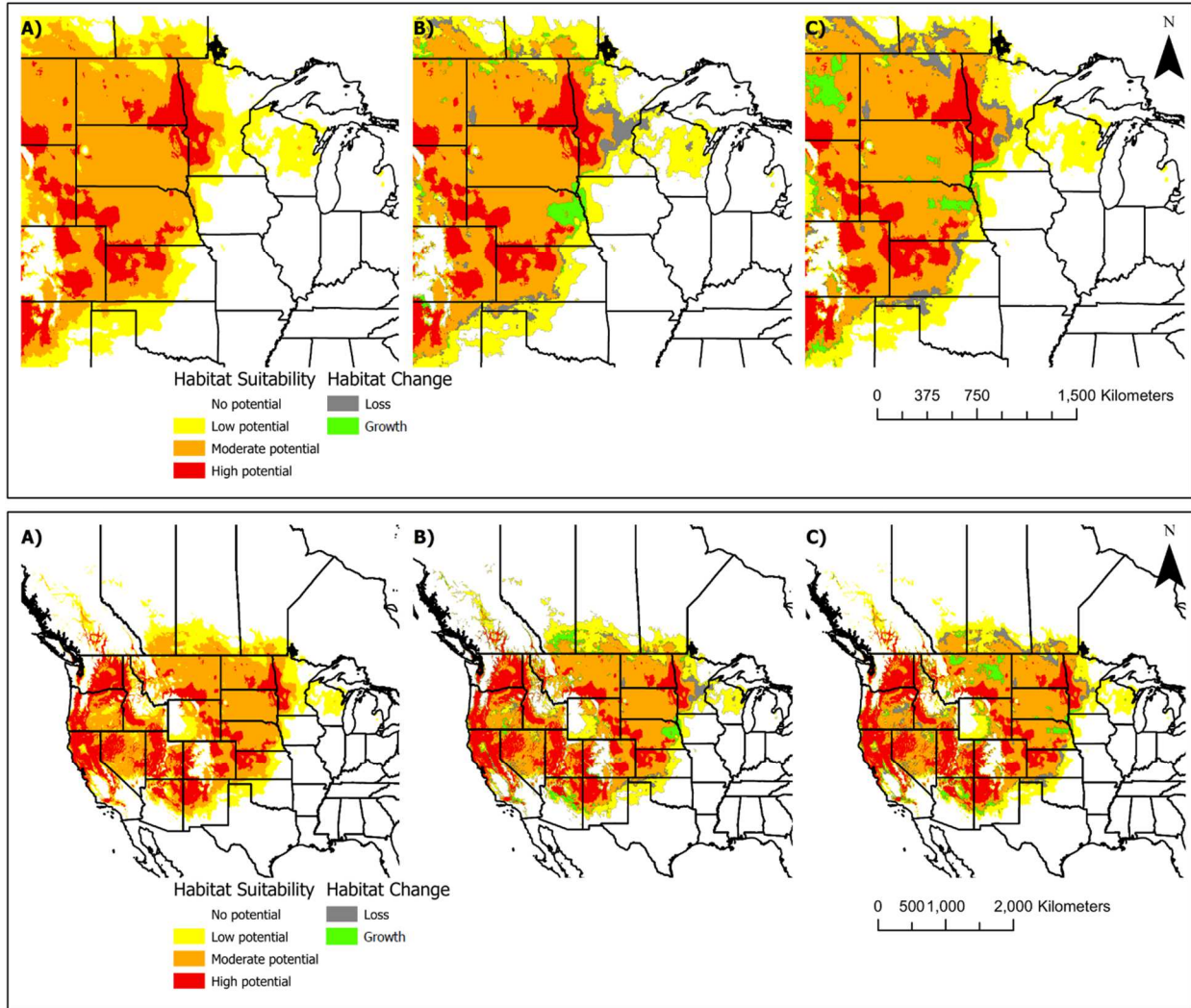
Figure B4. *Asclepias speciosa* (Showy milkweed) – LB-Forb – Region Map; *Asclepias speciosa* (Showy milkweed) – LB-Forb – Range Map.
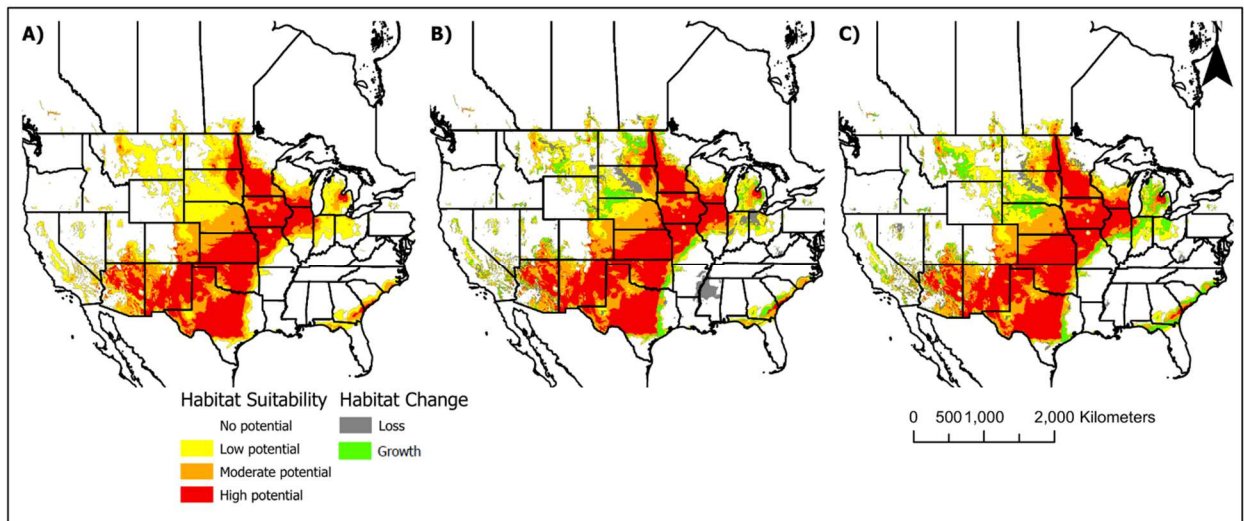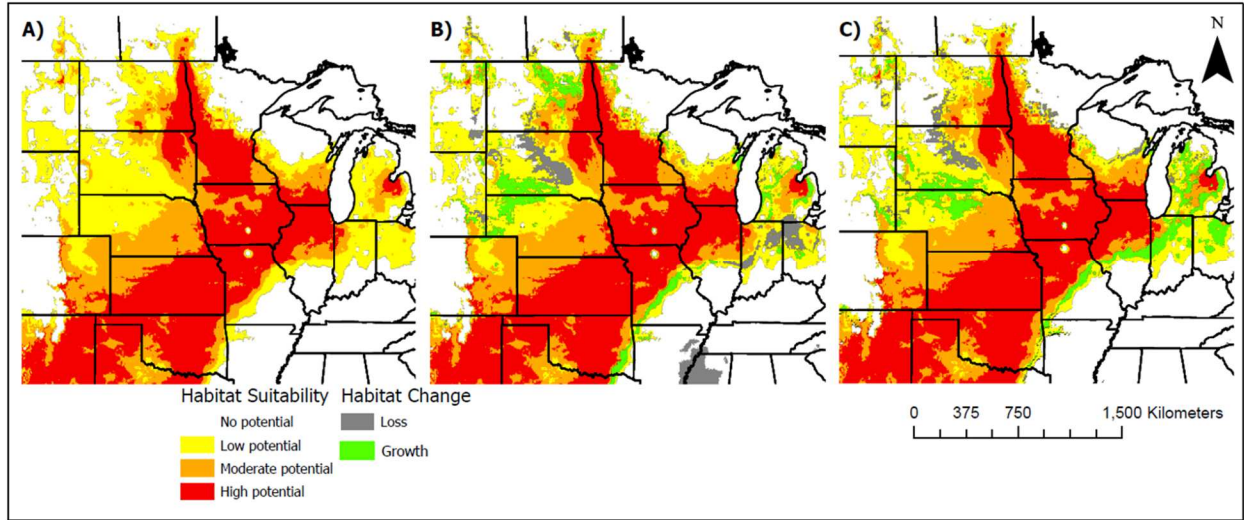
Figure B5. *Bouteloua curtipendula* (Sideoats grama) – WS-Grass Region Map; *Bouteloua curtipendula* (Sideoats grama) – WS-Grass Range Map.
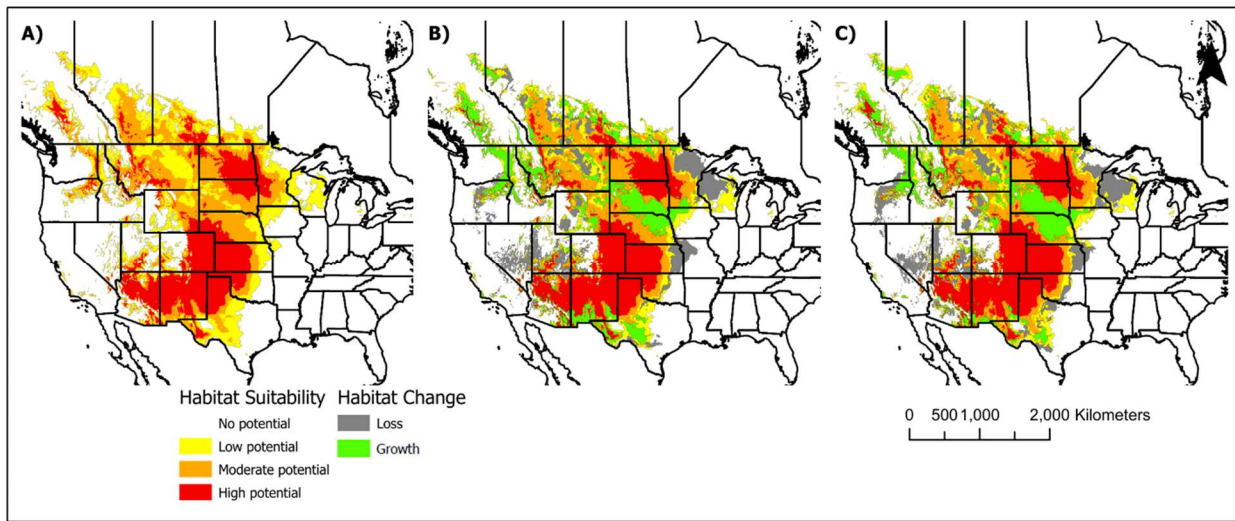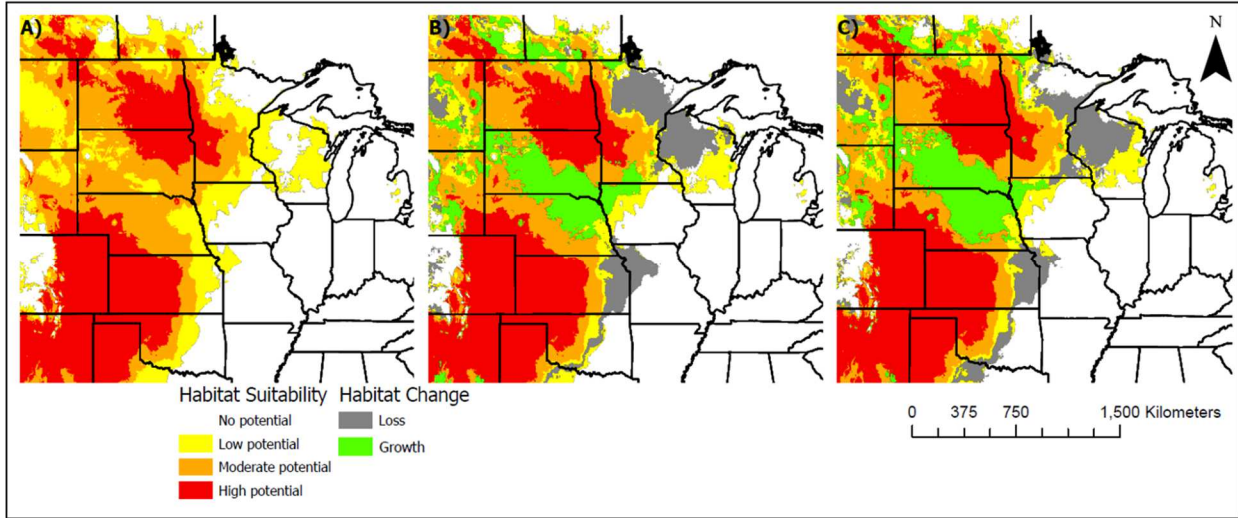
Figure B6. *Bouteloua gracilis* (Blue grama) – WS-Grass Range Map; *Bouteloua gracilis* (Blue grama) – WS-Grass Region Map.
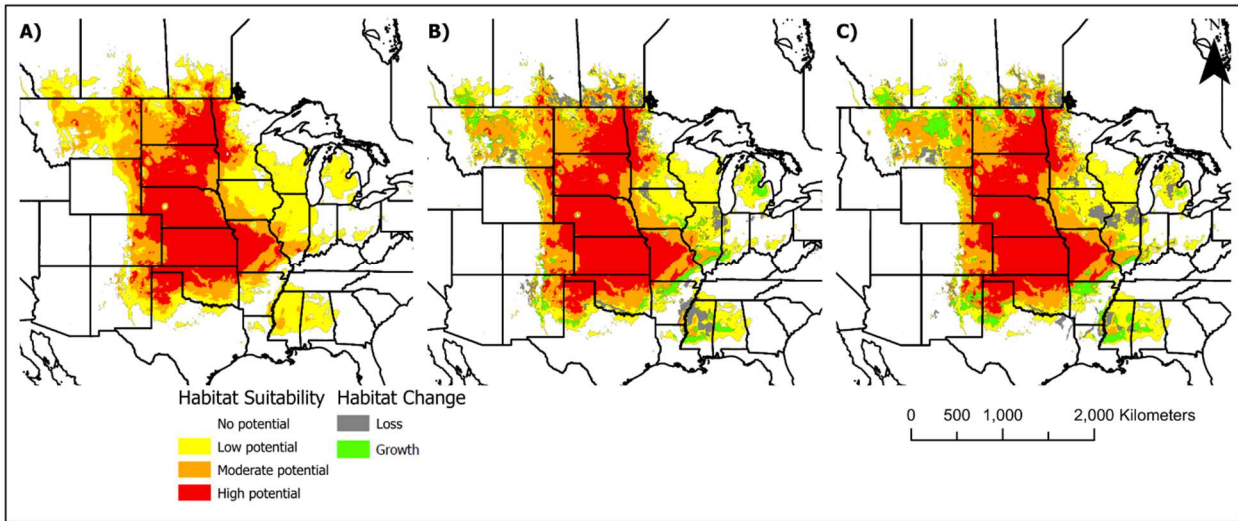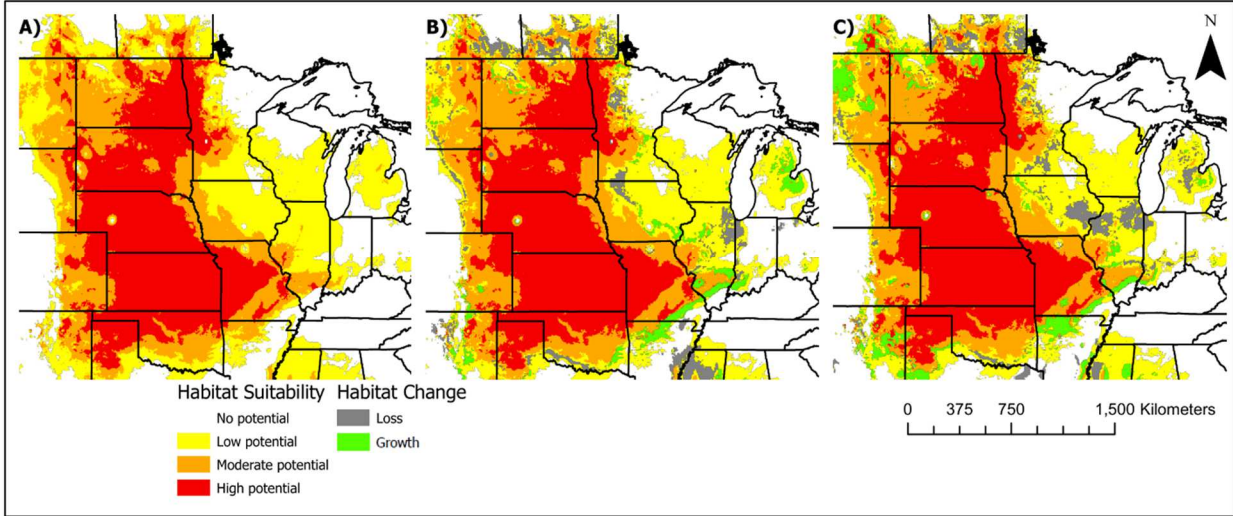
Figure B7. *Dalea purpurea* (Purple prairie clover) – Legume Region Map; *Dalea purpurea* (Purple prairie clover) – Legume Range Map.
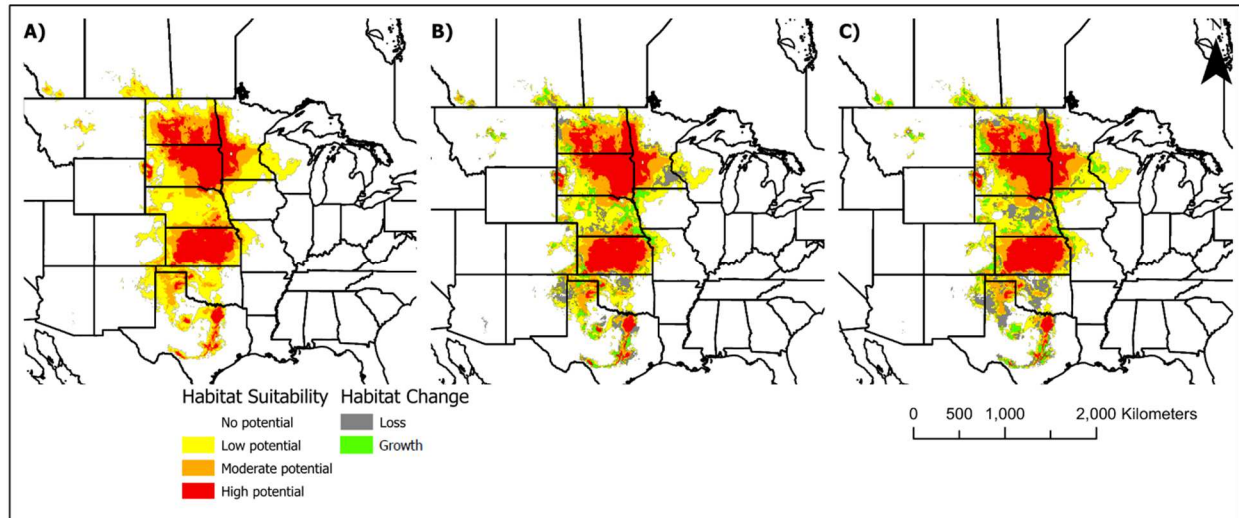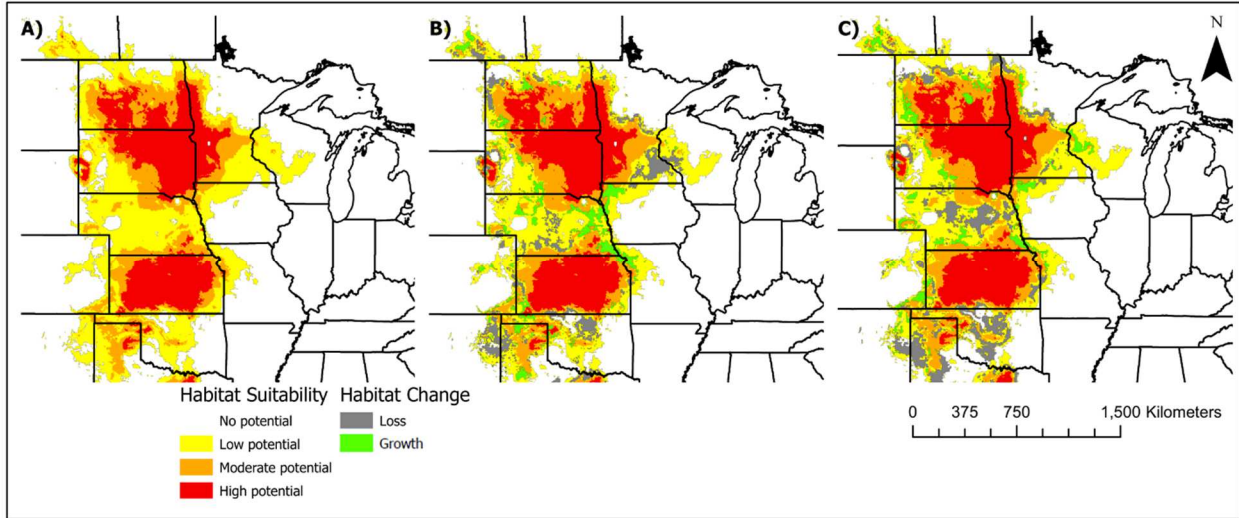
Figure B8. *Echinacea angustifolia* (Narrow-leaf coneflower) – Aster Region Map; *Echinacea angustifolia* (Narrow-leaf coneflower) – Aster Range Map.
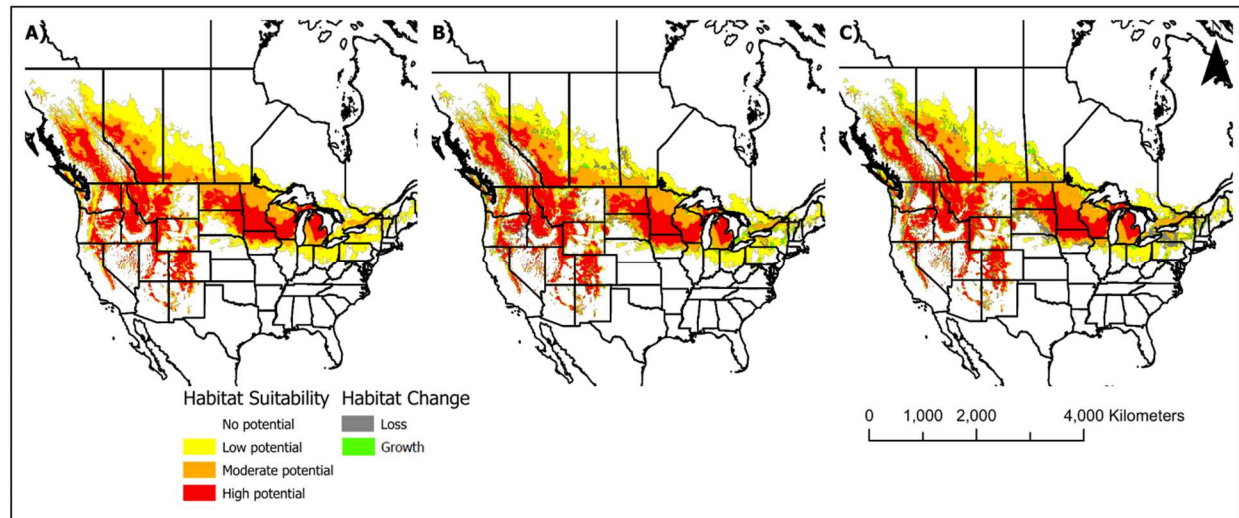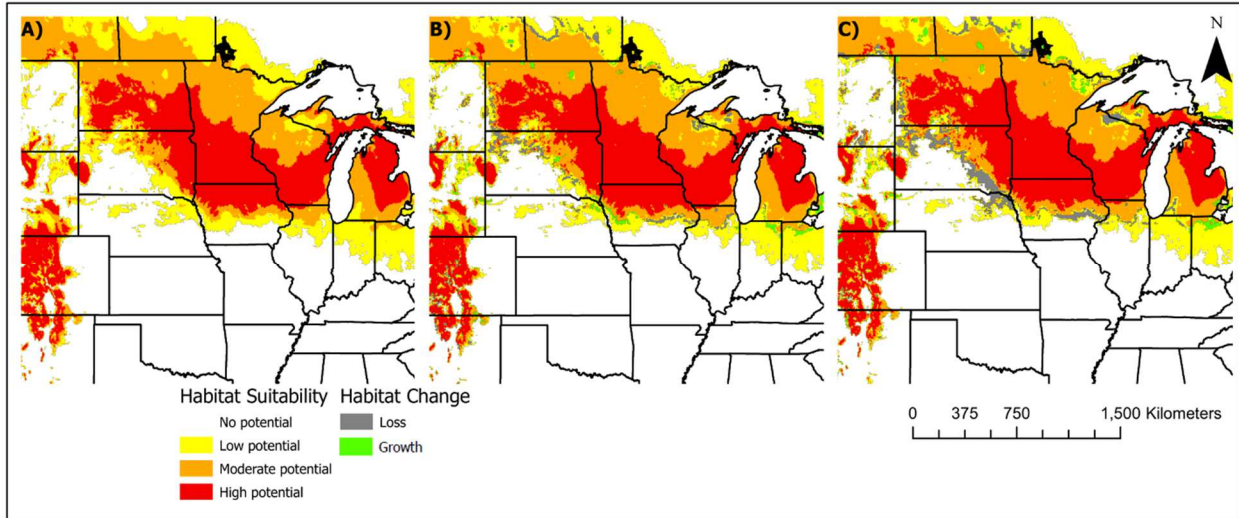
Figure B9. *Geum triflorum* (Prairie smoke) EB-Forb Region Map; *Geum triflorum* (Prairie smoke) EB-Forb Range Map.
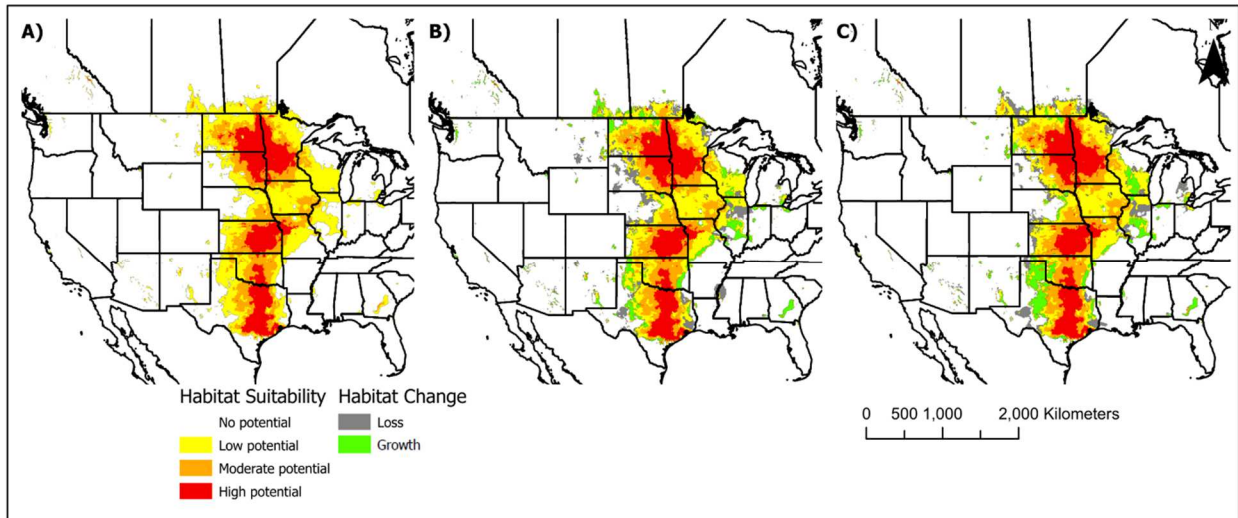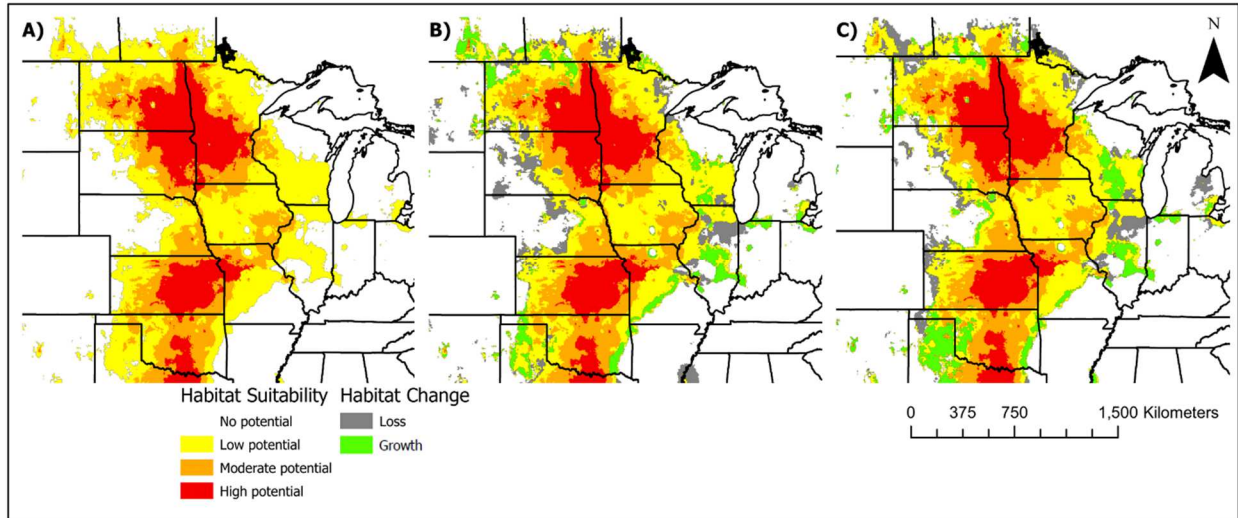
Figure B10. *Helianthus maximiliani* (Maximilian sunflower) – Aster Region Map; *Helianthus maximiliani* (Maximilian sunflower) – Aster Range Map.
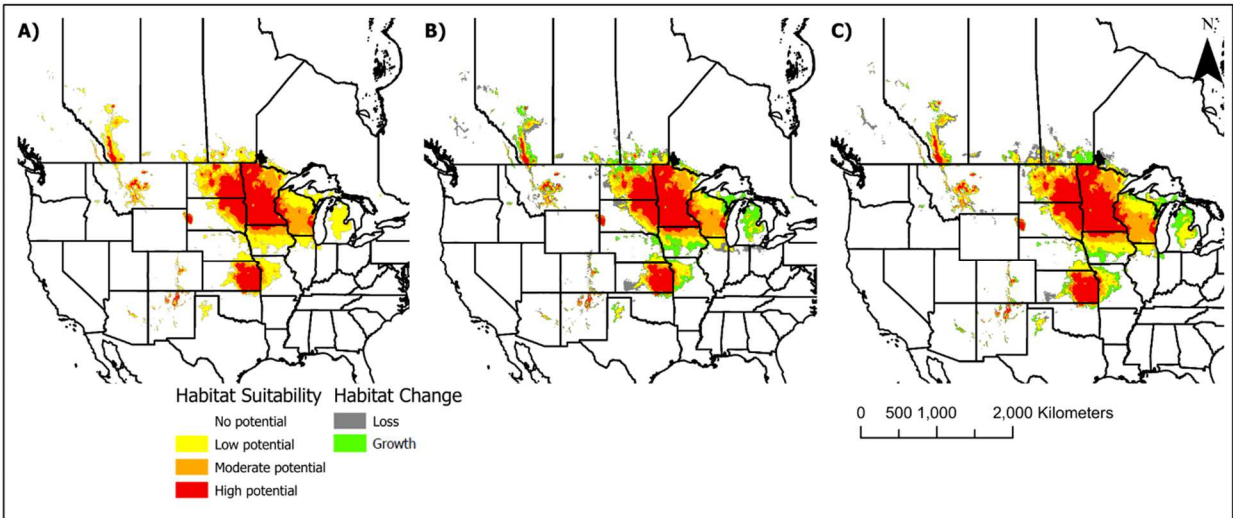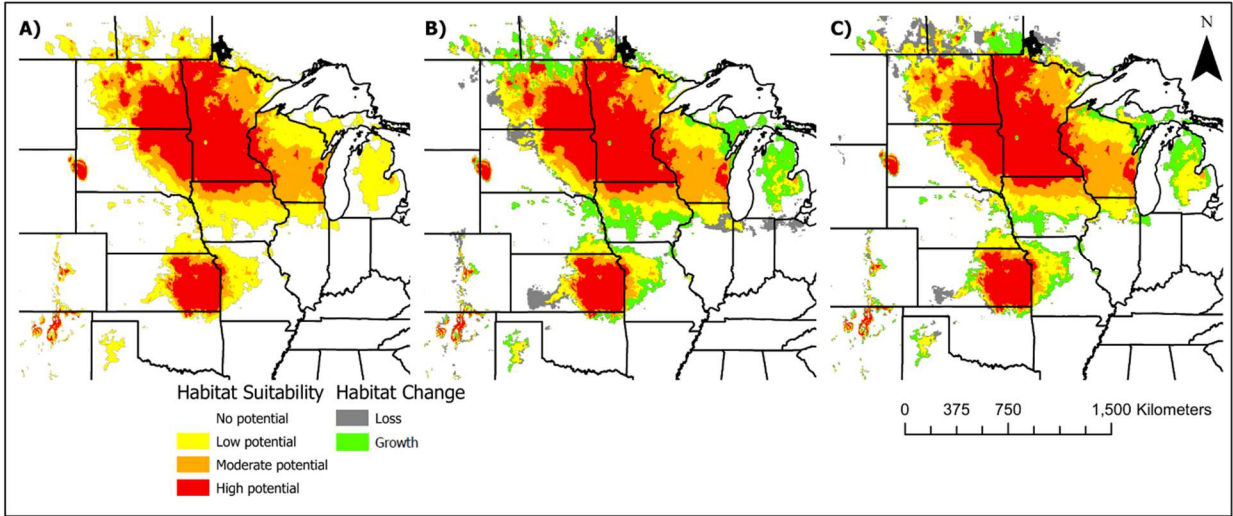
Figure B11. *Helianthus pauciflorus* (Stiff sunflower) – Aster Region Map; *Helianthus pauciflorus* (Stiff sunflower) – Aster Range Map.
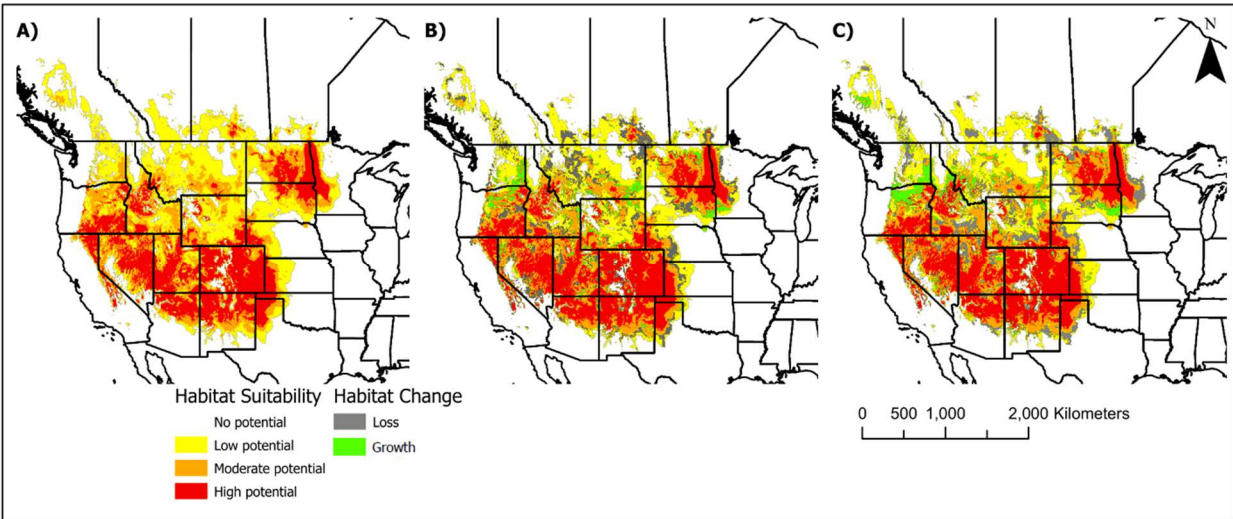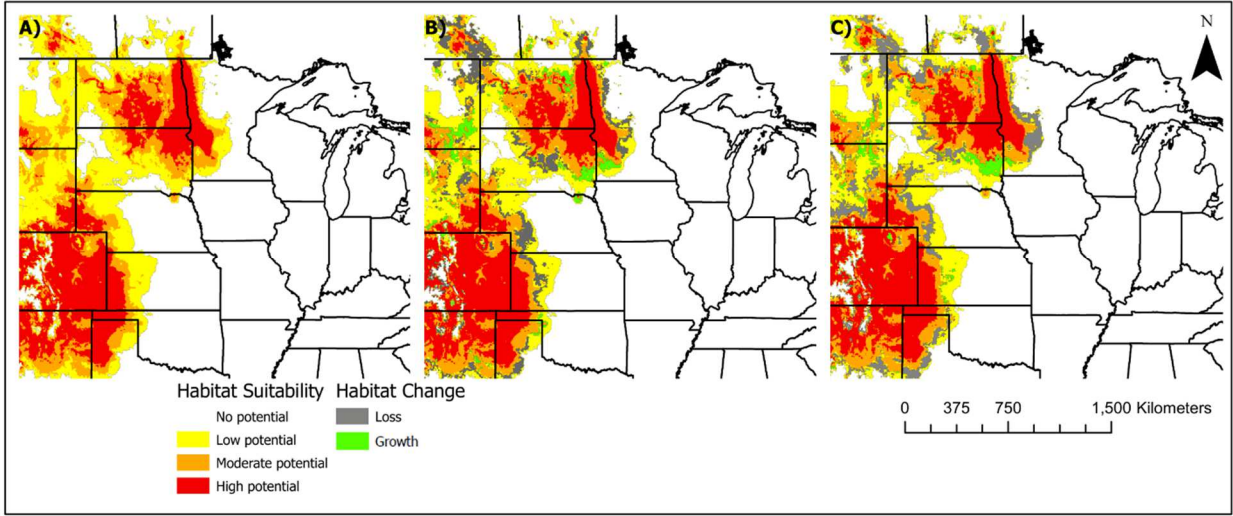
Figure B12. *Hespersostipa comata* (Needle and Thread grass) – CS-Grass Range Map;
Hespersostipa comata (Needle and Thread grass) – CS-Grass Region Map.
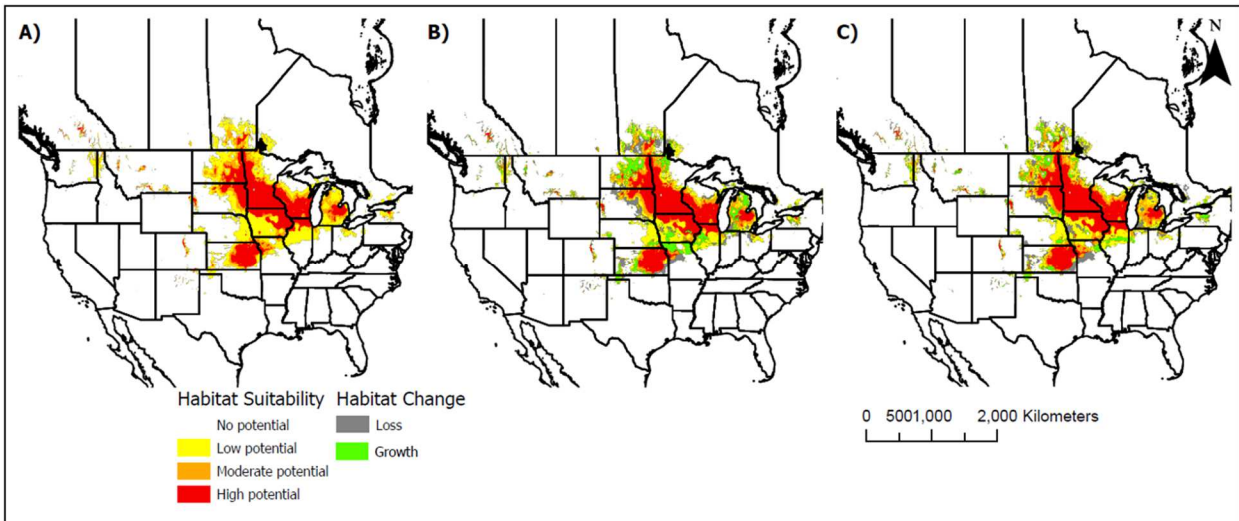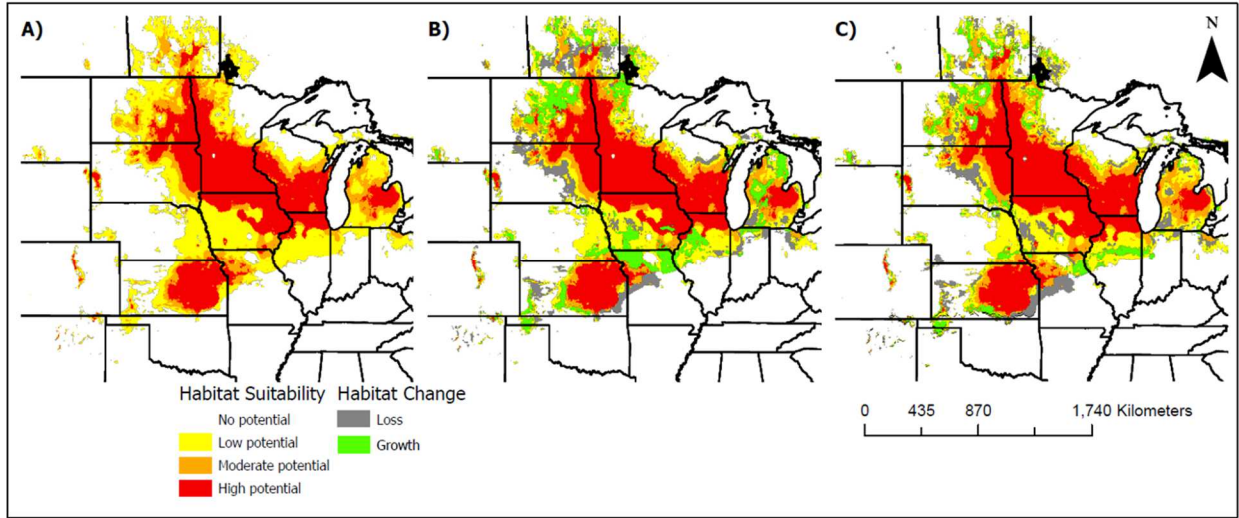
Figure B13. *Hespersostipa spartea* (Porcupine grass) – CS-Grass Region Map; *Hespersostipa spartea* (Porcupine Thread grass) – CS-Grass Range Map.
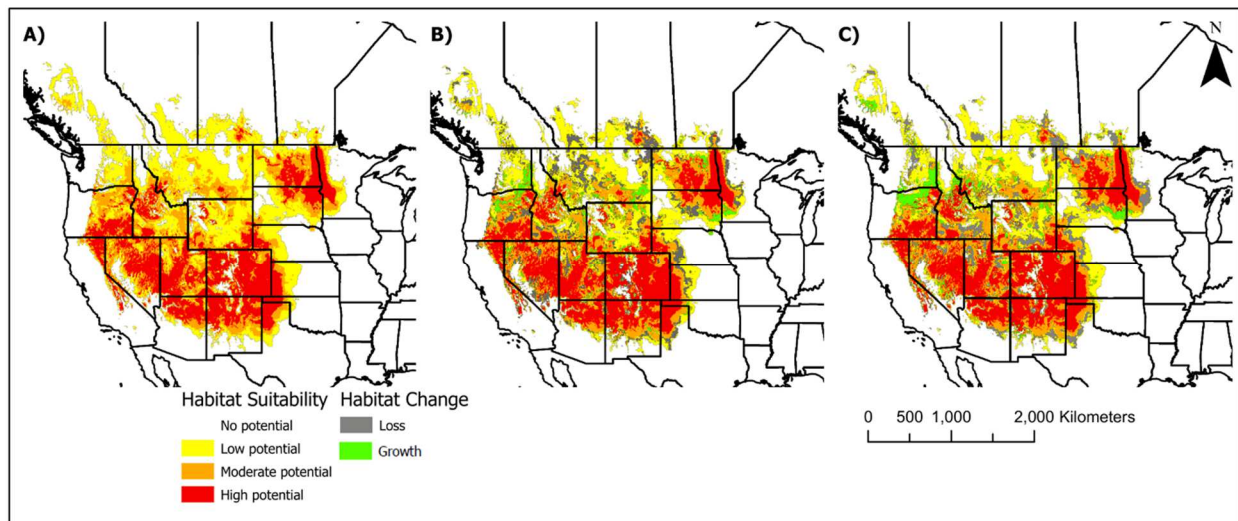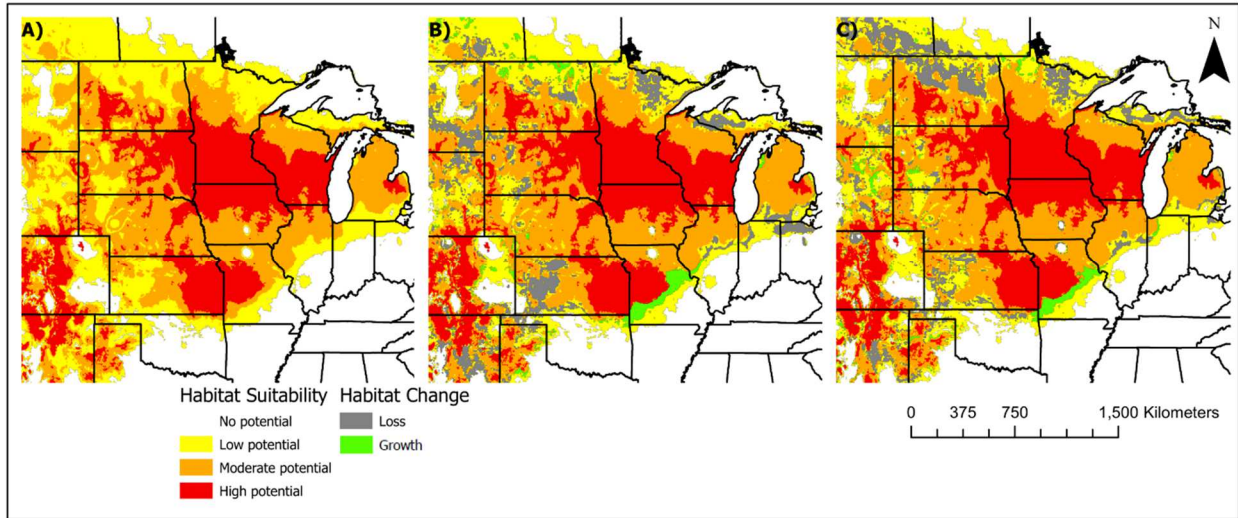
Figure B14. *Koeleria macrantha* (Junegrass) – CS-Grass Region Map; *Koeleria macrantha* (Junegrass) – CS-Grass Range Map.
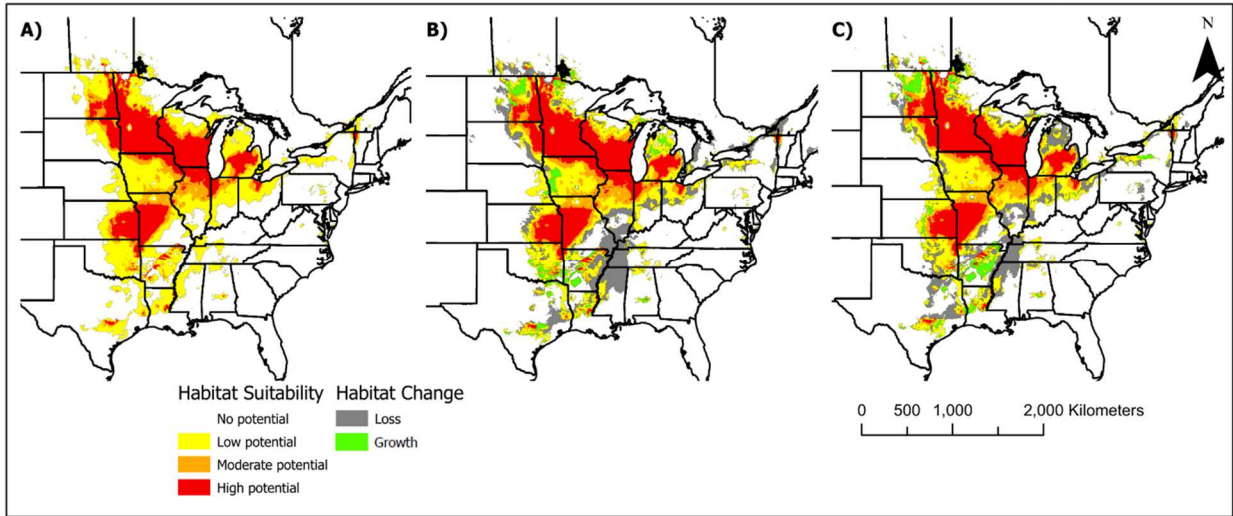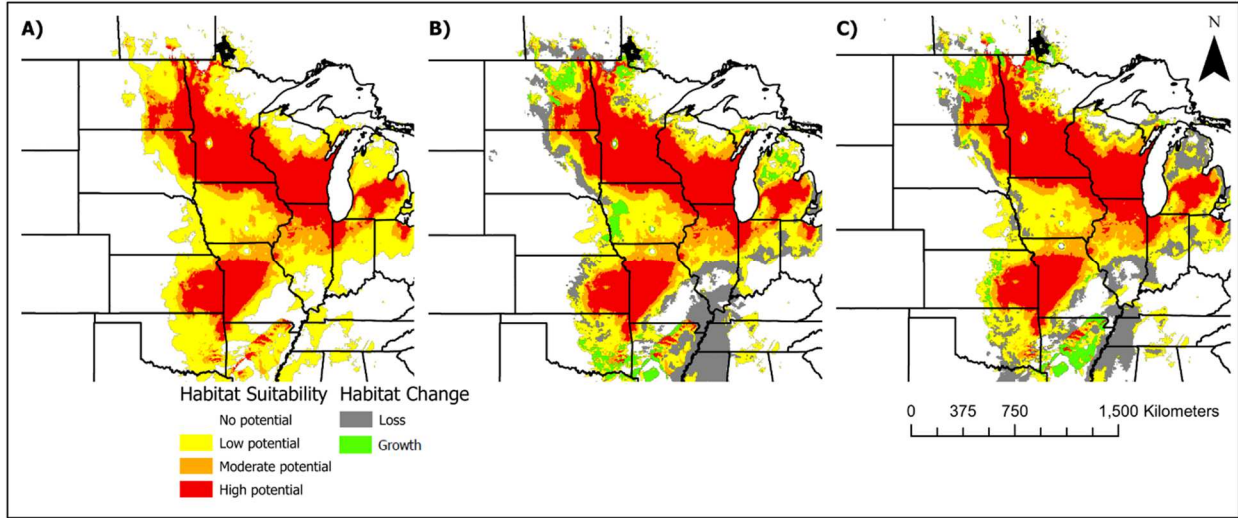
Figure B15. *Liatris aspera* – Aster Range Map; *Liatris aspera* – Aster Region Map.
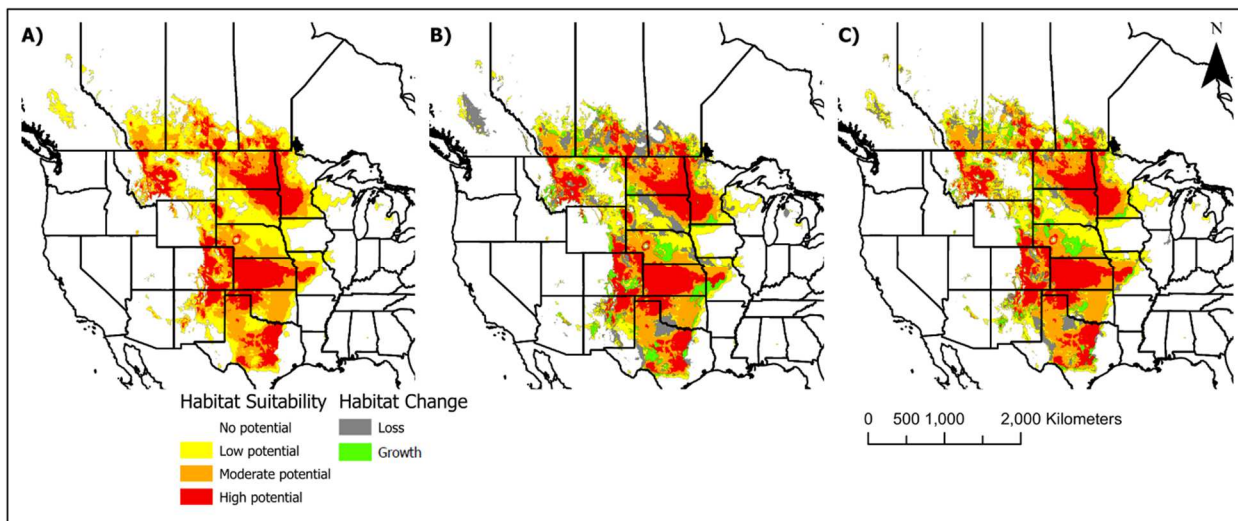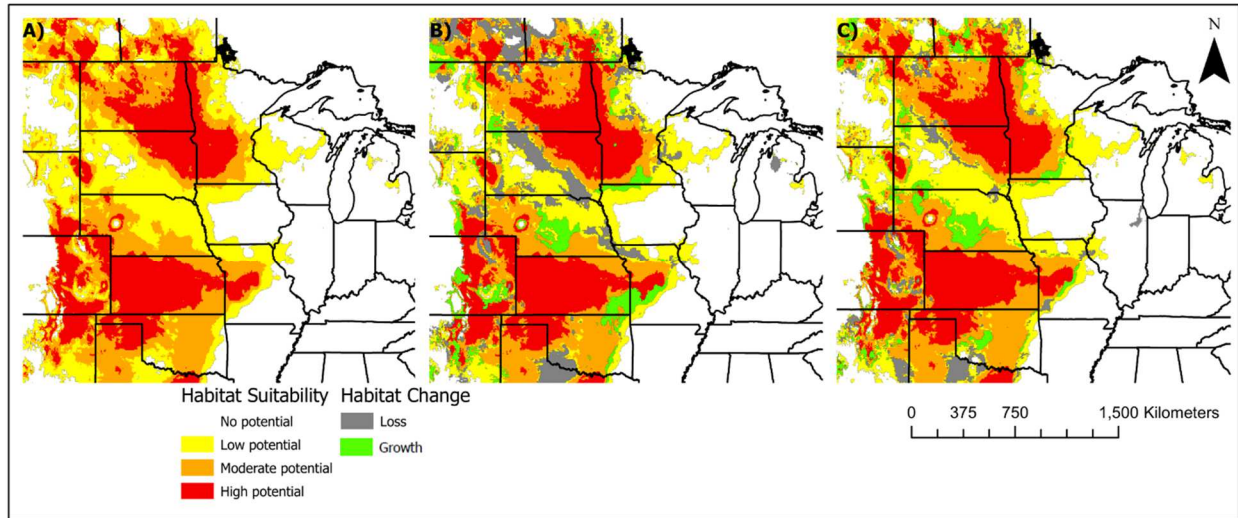
Figure B16. *Liatris punctata* – Aster Range Map; *Liatris punctata* – Aster Region Map.
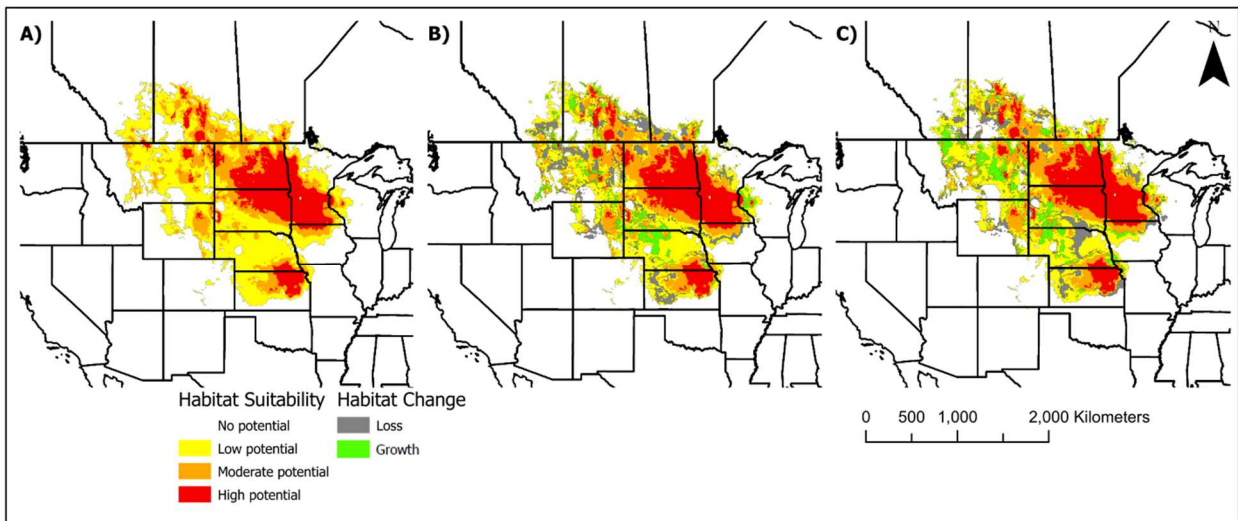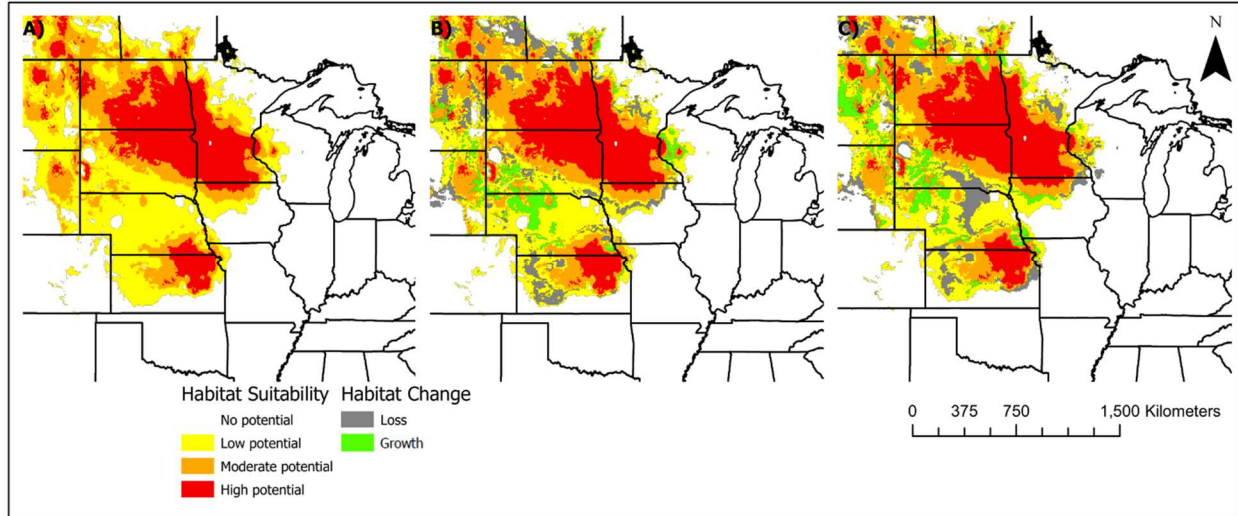
Figure B17. *Pediomelum argophyllum* – Legume Range Map; *Pediomelum argophyllum* – Legume Region Map.
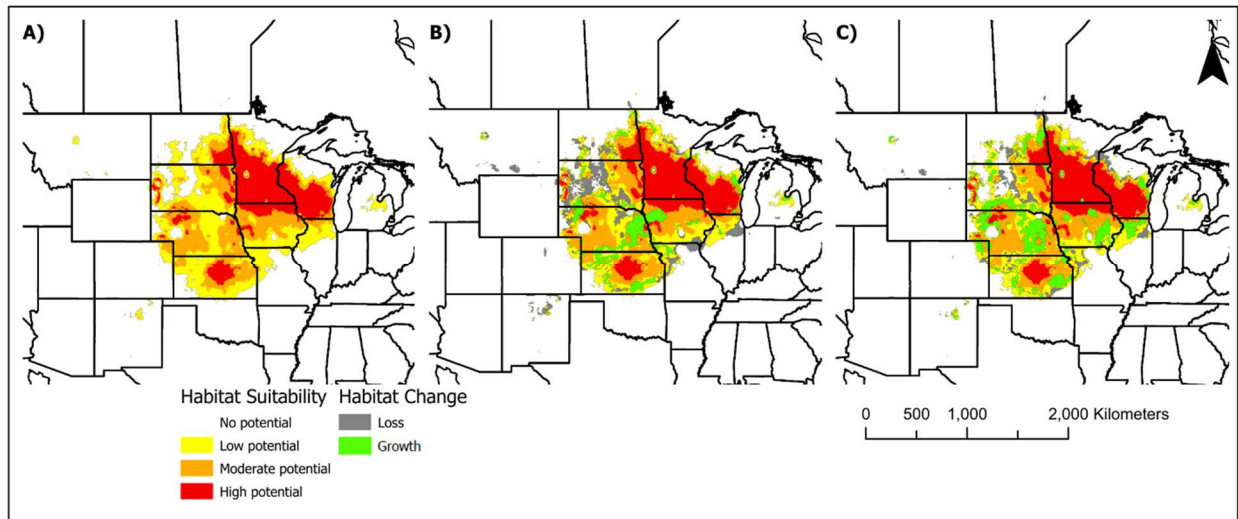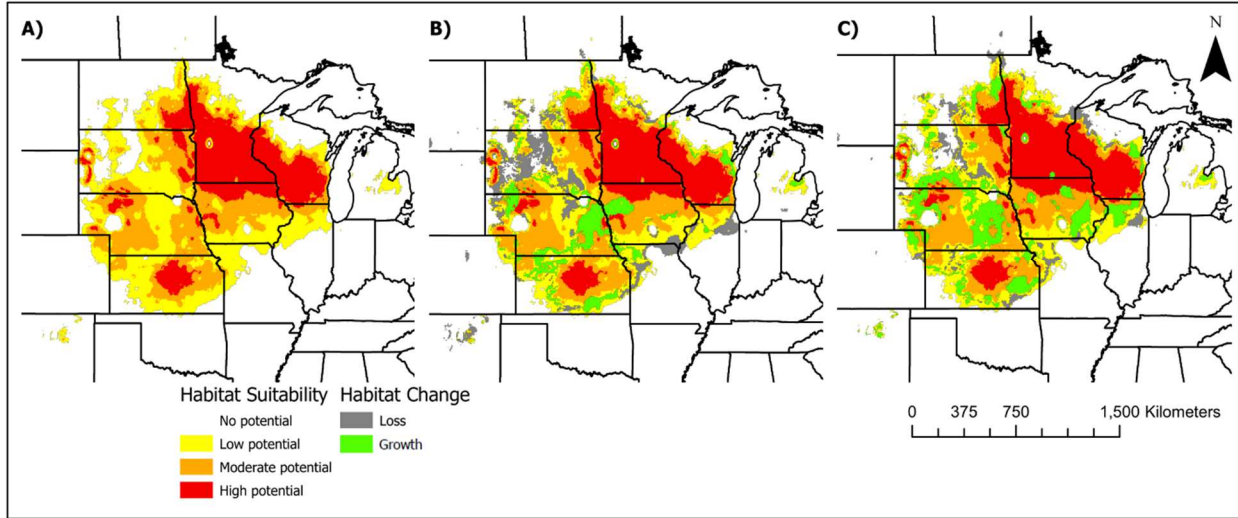
Figure B18. *Penstemon grandifloras* – EB- Forb Region Map; *Penstemon grandifloras* – EB-Forb Range Map.
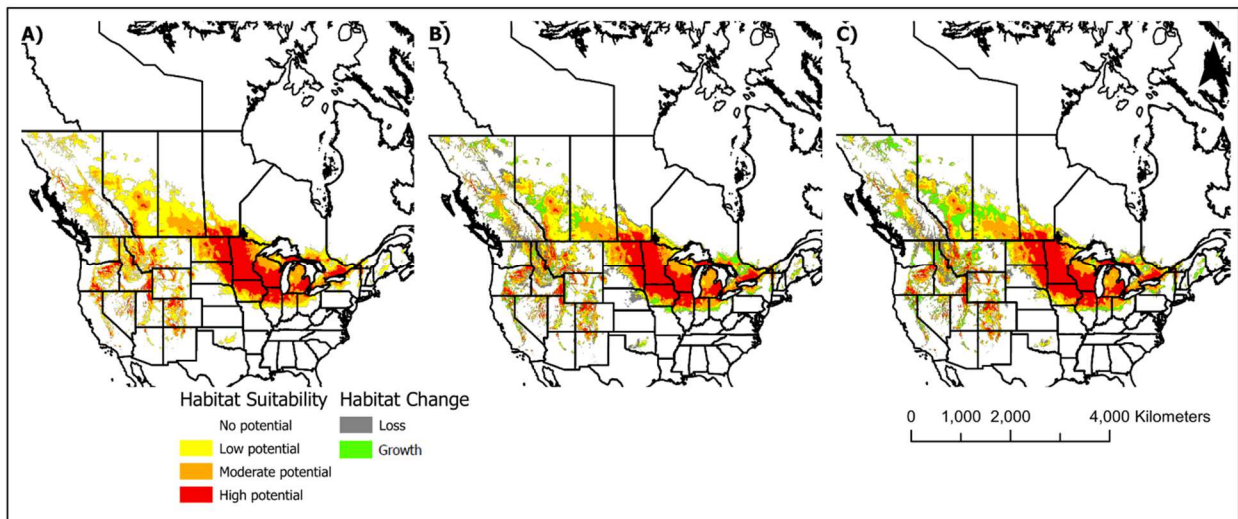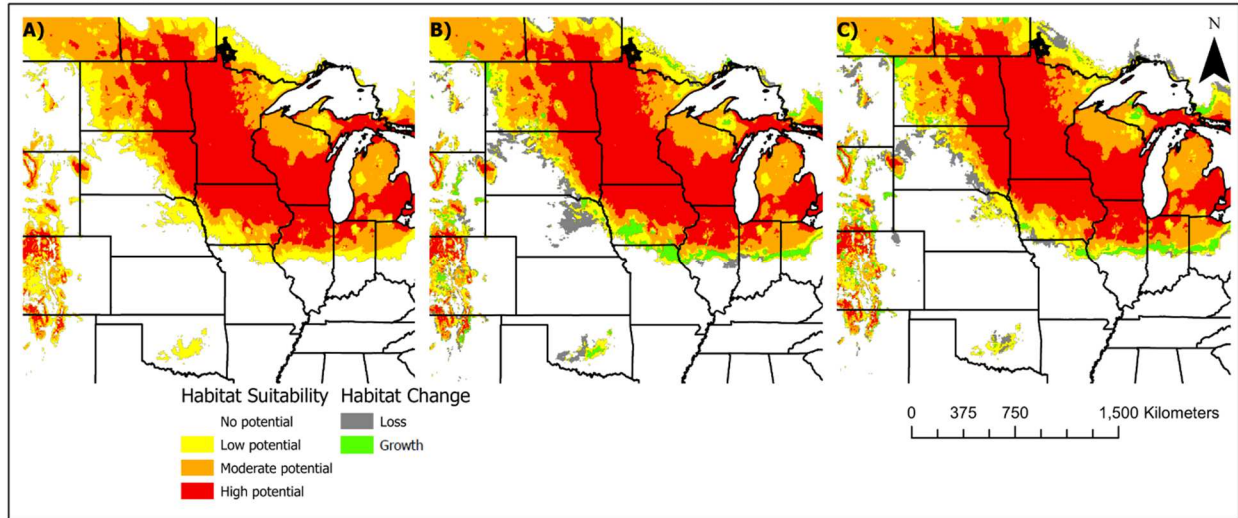
Figure B19. *Potentilla arguta* (Tall cinquefoil) – LB-Forb Region Map; *Potentilla arguta* (Tall cinquefoil) – LB-Forb Range Map.
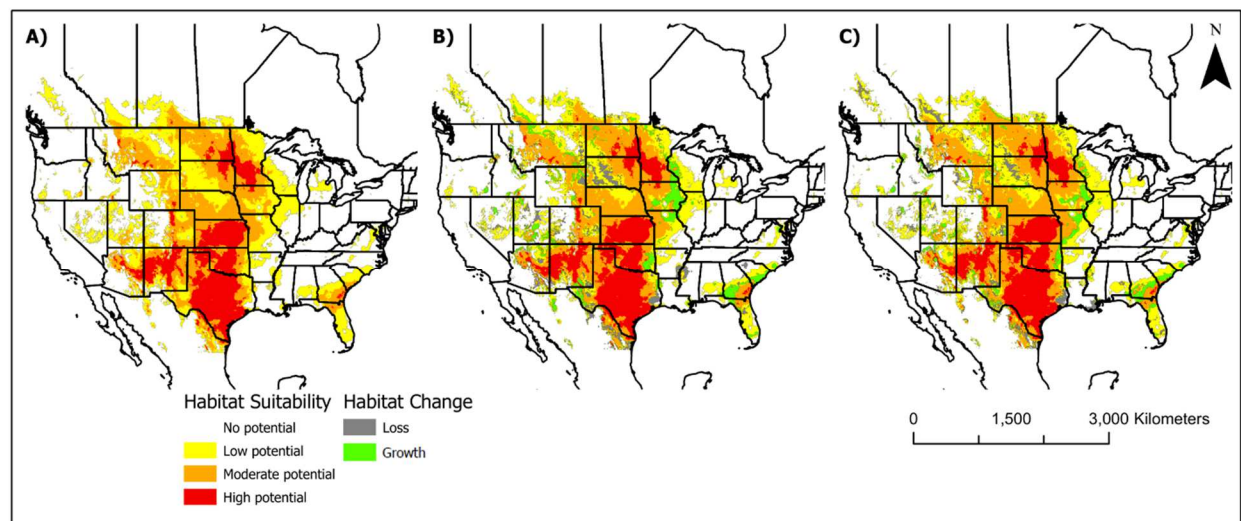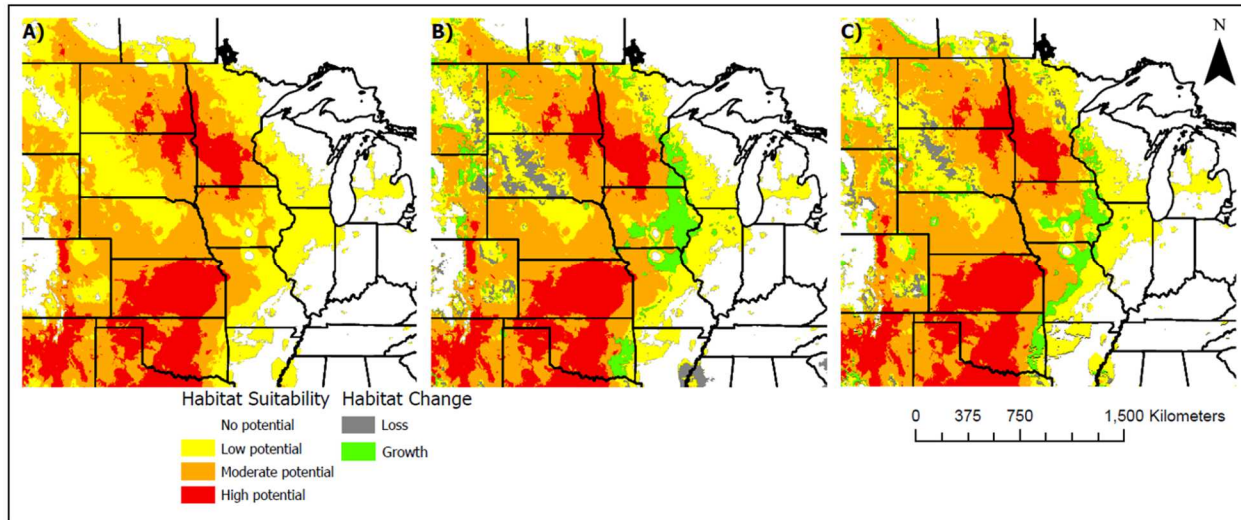
Figure B20. *Ratibida columnifera* (Prairie coneflower) – Aster Region Map; *Ratibida columnifera* (Prairie coneflower) – Aster Range Map.

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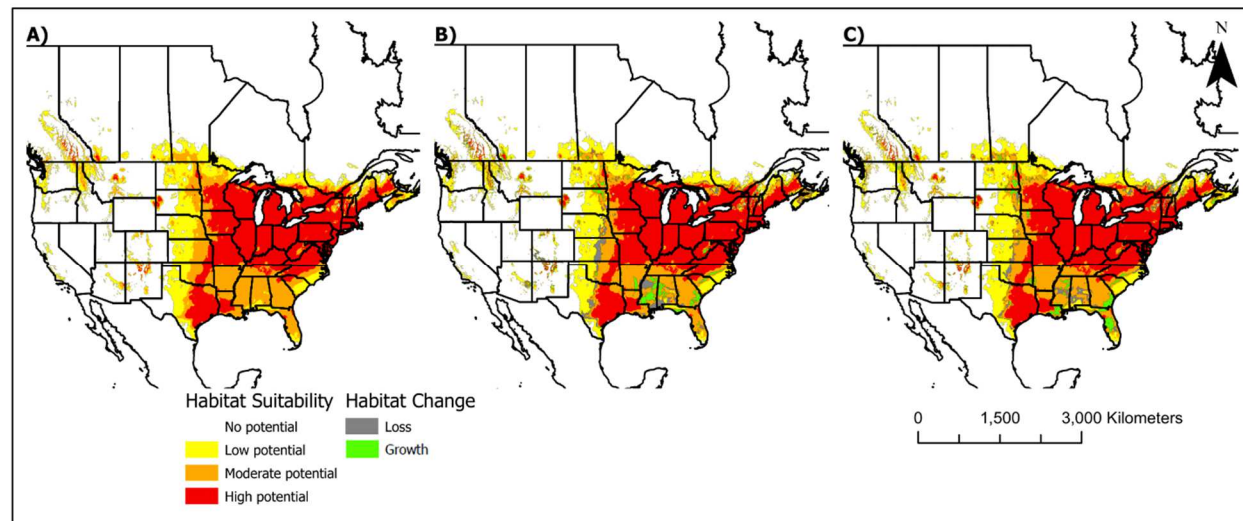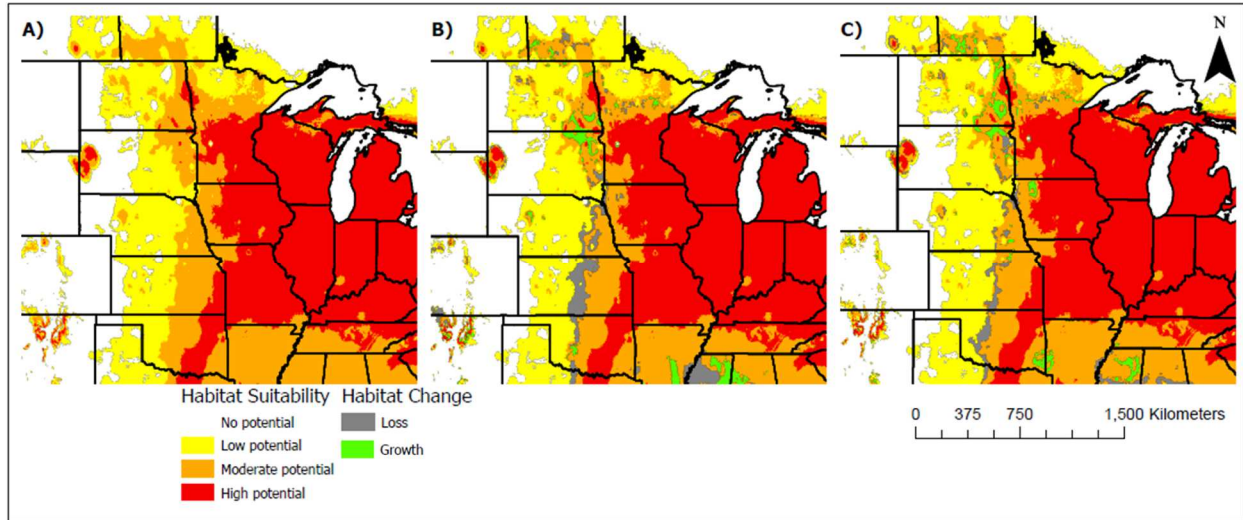
Figure B21. *Rudbeckia hirta* (Black-eyed Susan) – Aster Region Map; *Rudbeckia hirta* (Black-eyed Susan) – Aster Range Map.
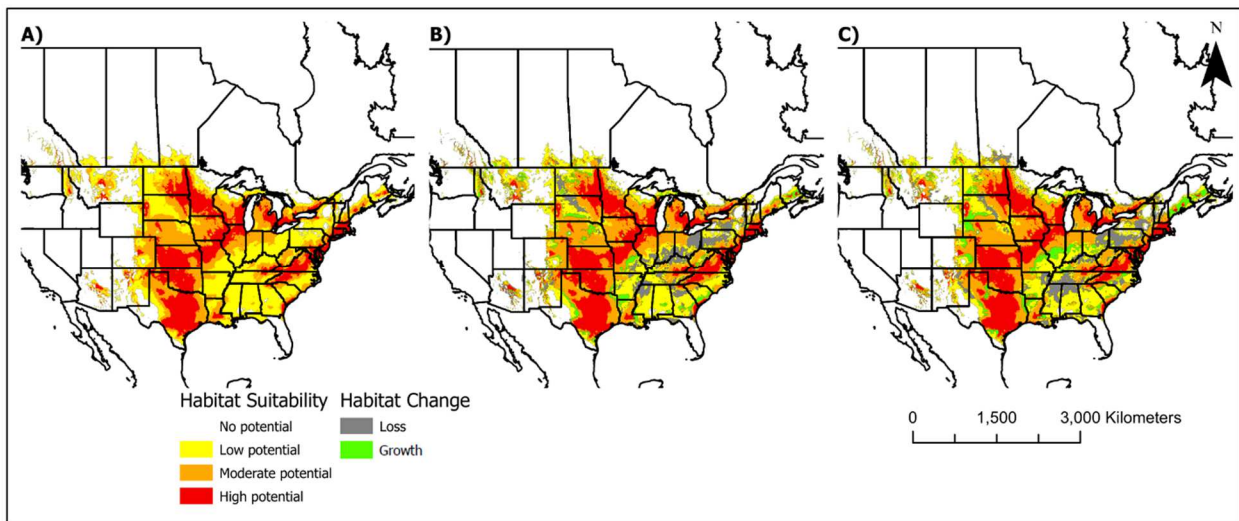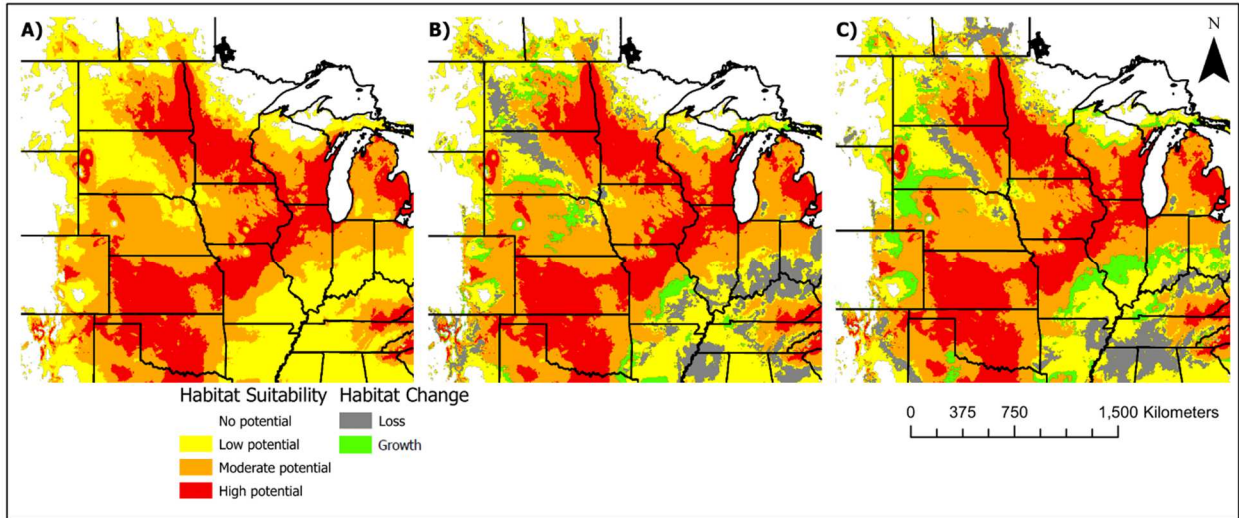
Figure B22. *Schizachyrium scoparium* (Little bluestem) – WS-Grass Region Map;
Schizachyrium scoparium (Little bluestem) – WS-Grass Range Map.
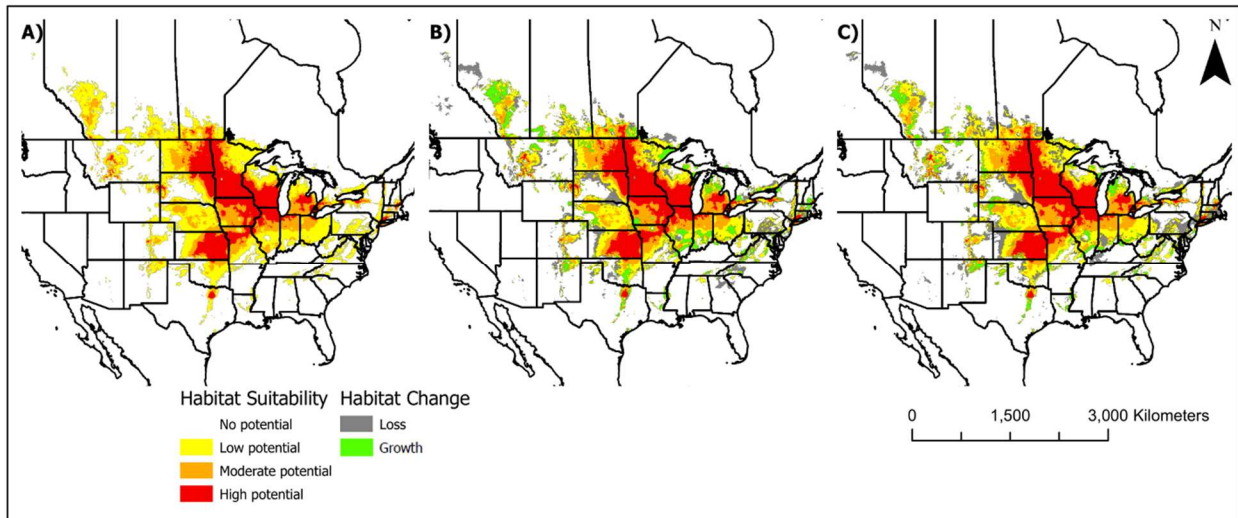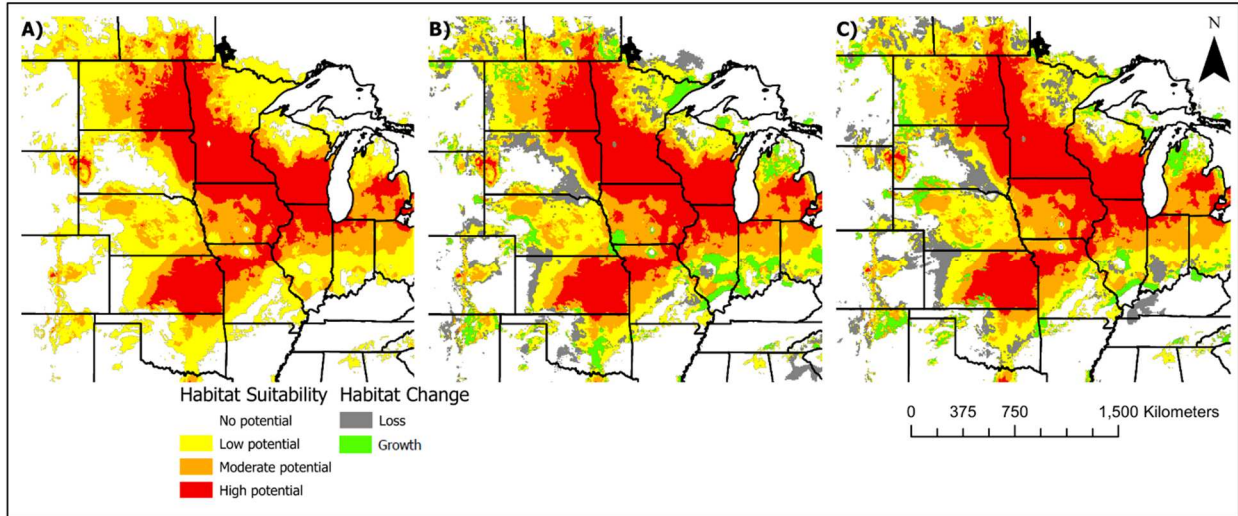
Figure B23. *Solidago rigida* (Stiff goldenrod) – Aster Region Map; *Solidago rigida* (Stiff goldenrod) – Aster Range Map.
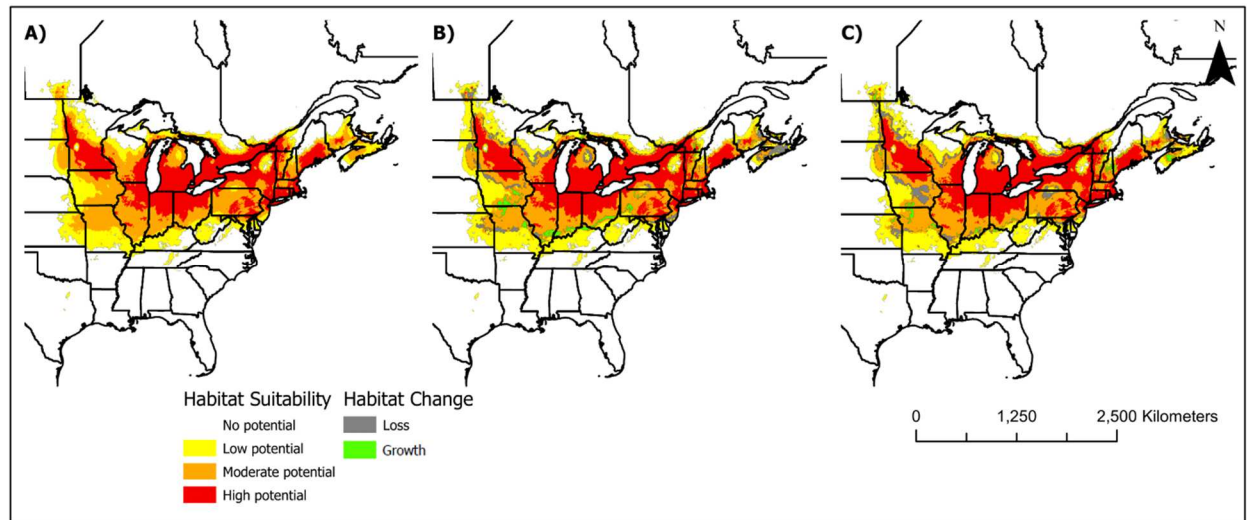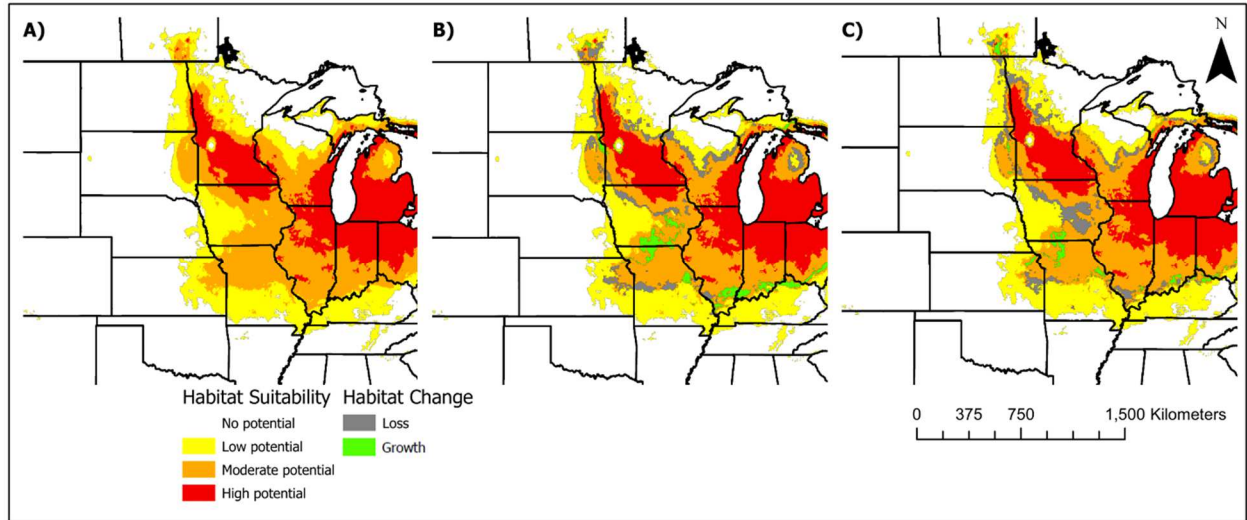
Figure B24. *Symphyotrichum novae-angliae* (New England aster) – Aster Region Map; *Symphyotrichum novae-angliae* (New England aster) – Aster Range Map.
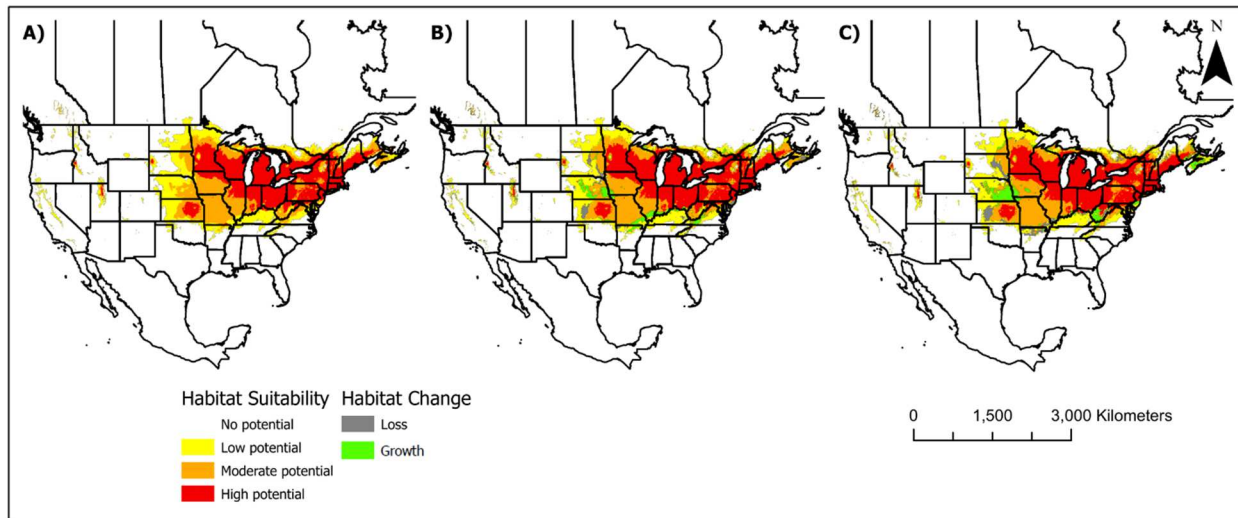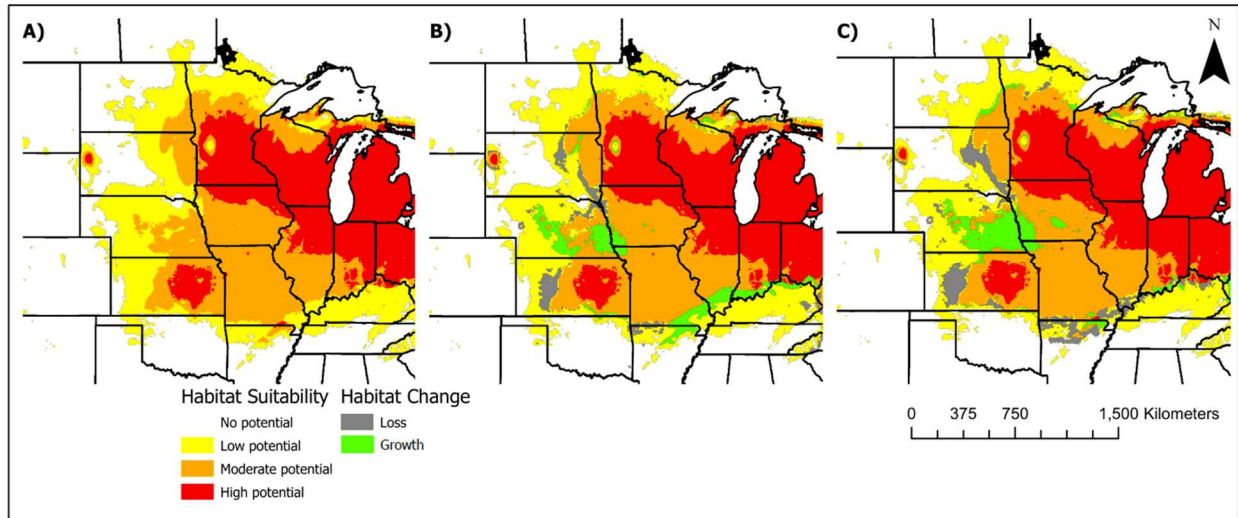
Figure B25. *Verbena hastata* (Blue Verbena) – LB-Forb Region Map; *Verbena hastata* (Blue Verbena) – LB-Forb Range Map.
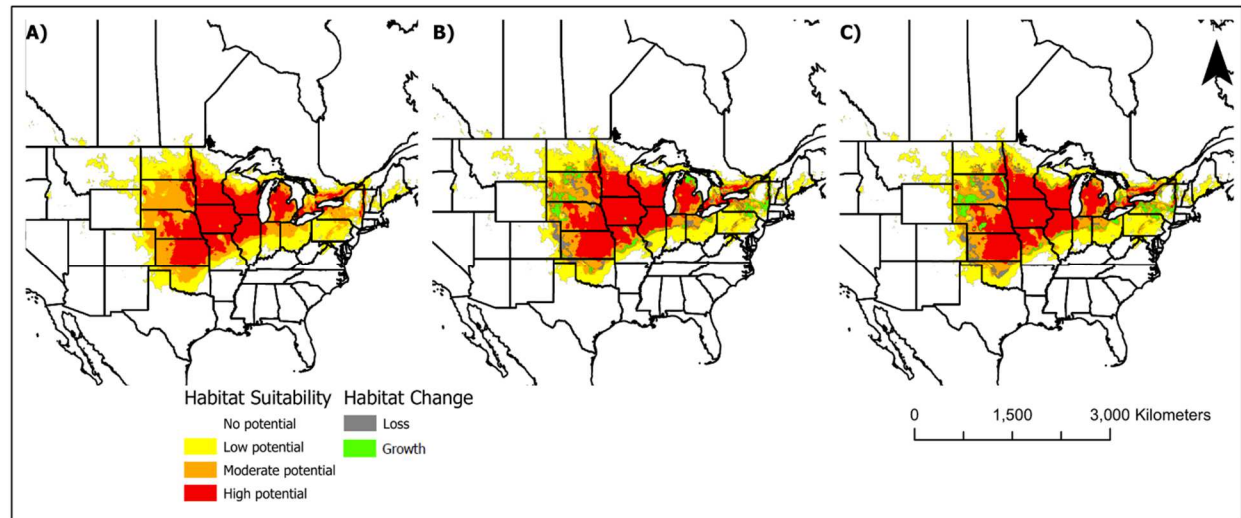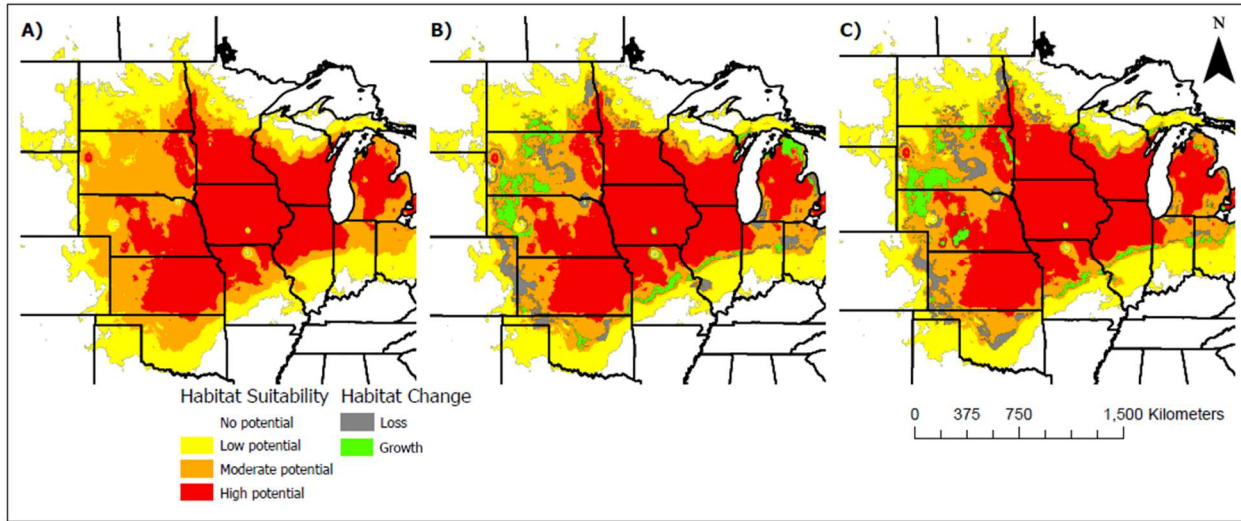
Figure B26. *Verbena stricta* (Hoary Verbena) – LB-Forb Region Map; *Verbena stricta* (Hoary Verbena) – LB-Forb Range Map.