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Examining Biodiversity Metrics and the Utility of DNA Barcoding in the Northern Great Plains

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EXAMINING BIODIVERSITY METRICS AND THE UTILITY OF DNA BARCODING IN THE NORTHERN GREAT PLAINS

BY
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THESIS ACCEPTANCE PAGE

Sarah Herzog

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Dean, Graduate School Date

This thesis is dedicated to the strong women that surround and inspire me. To my mom, Melissa, who has always supported me and my endeavors, regardless of what it was (or how bad it smelled). To my grandmothers, Shirley and Margaret, that took me outside to look for bugs and poke at pond scum. To my advisor, Maribeth, for being a prime example of a female scientist. To my gal pals, for reminding me that female friends are the best of friends. And to all the women in botany and natural resource management that came before me and broke through the glass ceiling. And lastly, my dad, Steve, for believing in my strength and raising a river rat as a daughter.

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South Dakota State University is located on the ancestral territory of the Oceti Sakowin [oh-CHEH-tee SHAW-koh-we], meaning Seven Council Fires which is the proper name for the people commonly called Sioux. The tribal alliance, made up of individual bands, of the Seven Council Fires is based on kinship, location and dialects: Santee-Dakota, Yankton-Nakota and Teton-Lakota. The seven tribes now occupy nine reservations in South Dakota: Cheyenne River Sioux Tribe, Crow Creek Sioux Tribe, Flandreau Santee Sioux Tribe, Lower Brule Sioux Tribe, Oglala Sioux Tribe, Rosebud Sioux Tribe, Sisseton-Wahpeton Oyate, Standing Rock Sioux Tribe and Yankton Sioux Tribe.

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Abstract

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Due to heavy threats to remaining global floral and faunal diversity, it is imperative we can identify species and quantify ecosystem health to find best practices for land management and conservation. The tallgrass prairies of the Northern Great Plains are one example of a heavily imperiled ecosystem. The tallgrass prairies have been reduced to less one percent of their historical extent and are facing continued loss. Genetic approaches and evolutionary theory offer insights for identifying species and assessing how biodiversity metrics may correlate with ecosystem processes. My two projects aim to address two facets imperative to conservation in the tallgrass prairie plant communities of the Prairie Coteau, a region defined by its relatively higher elevation to surrounding: 1) identification of regional flora using DNA barcodes and 2) incorporating evolutionary history (phylogenetic diversity, PD) into biodiversity assessments of plant communities. I assessed high throughput sequencing methods for DNA barcoding using four DNA regions and evaluated their success in identifying species occurring at Oak Lake Field Station at South Dakota State University. I found moderate success for species level identification and high success rates for genus and family level identifications. Including multiple loci resulted in higher success than one-locus barcodes. Phylogenetic diversity was compared to species richness by taking floristic surveys of 21 sites across the Prairie Coteau. PD only weakly correlated with species richness. Many of the sites sampled experienced lower PD than expected. My results indicate 1) DNA barcoding has potential to act as an extension service for regional plant identification, and 2) increasing species richness is not sufficient when aiming to maximize PD.

CHAPTER 1: INTRODUCTION

Healthy ecosystems are vital for the long term survival of species and ecosystem functioning. Biodiversity impacts aquatic and terrestrial processes, with many studies finding higher diversity produces better functioning systems (Tilman 1996, Tilman et al. 2006, Cardinale et al. 2012, Cadotte 2013). Additionally, higher species diversity increases the ability of a system to withstand invasion and disturbance, including climate change (Davies et al. 2011, Isbell et al. 2015).

Humans benefit from numerous ecological processes. The Millenium Ecosystem Assessment (2005) defined these benefits to humans, ecosystem services, as belonging in three categories: provisioning (production of food, water, wood, timber, and fiber), regulating (affecting climate, floods, disease, wastes, and water quality), cultural (recreational, aesthetic, and spiritual), and supporting (soil formation, photosynthesis, and nutrient cycling). Protection of these services is imperative for the continued health of humans and the natural world (Cardinale et al. 2012). A 2019 UN report (Díaz et al. 2019) shows just how dependent humans are on ecosystems. Two billion people rely on wood fuel as their primary energy, and four billion people rely on natural medicines and numerous drugs are natural or based on natural products. Additionally, more than 75 percent of global food crop types are reliant on animal pollination.

Evidence suggests reducing biodiversity in an ecosystem results in reduced services, such as biomass production (Naeem 1994, Tilman 1996, Hector et al. 1999). Additionally, high levels of plant diversity have been shown to lead to higher carbon storage (Hungate et al. 2017, Binder et al. 2018). Recent estimates place ecosystem service contributions globally at US$125 trillion per year with US$24 trillion per year in the Americas alone (Costanza et al. 2014, IPBES 2018, World Wildlife Fund 2018a). Staggering losses of diversity have already occurred, with 60 percent population declines in animals over the last 40 years, and these

losses have profound impacts on the functioning of global natural processes (World Wildlife Fund 2018a). By one estimate, 65% of ecosystem services are in decline, with 21% declining sharply (IPBES 2018). The situation becomes more dire when evidence indicates increased rate of species loss with ongoing species loss (Hooper et al. 2012).

IPEB's 2019 Global Assessment Report on Biodiversity and Ecosystem Services indicates the massive amount of biodiversity lost and threatened globally (Díaz et al. 2019). The report shows terrestrial native species abundances have fallen, at minimum, 20 percent since 1900, and population loss is likely accelerating. Additionally, 75 percent of Earth's land surface has been significantly altered by human activity, leading to lost productivity in 23 percent of terrestrial systems. An average of 25 percent of the assessed plant and animal groups were threatened, suggesting nearly one million species are facing extinction, many of those within decades if current trends in resource exploitation are maintained. If no action is taken, it is likely the global extinction rate will continue to increase; the current rate is already estimated to be "at least tens to hundreds of times higher than it has averaged over the past 10 million years" (Díaz et al. 2019).

Of the many ecosystems under threat, grasslands are particularly impacted. Temperate grasslands are one of the most altered biomes globally and the least protected (Hoekstra et al. 2005, Peart 2008, Newbold et al. 2016). Grasslands cover about eight percent of terrestrial surfaces, occurring on every continent except Antarctica (Peart 2008). Between 1700 and 1992, approximately 20 percent of global grasslands were converted, with conversion rates in the United States substantially greater than the cumulative global average during this period, resulting in a nearly 50 percent loss of grasslands in the United States (Ramankutty and Foley 1999).

Once covering an extensive amount of North America in the United States and Canada, the native grassland communities of the Great Plains have seen massive losses in extent. Only around half of these communities remain, and of

the areas that do remain, 87 percent of those are on poor and marginal quality soils (World Wildlife Fund 2018b). Additionally, in recent years the Great Plains have lost a greater proportion of intact grassland than that of the Brazilian Amazon rainforest (World Wildlife Fund 2018b). Although all native ecosystems in the Great Plains have suffered extensive range loss, none of its habitat types have experienced as drastic of losses as the tallgrass prairie (Samson et al. 2004). Rates of loss have been particularly high within the Western Corn Belt (WCB) of the United States, where over 99 percent of pre-settlement tallgrass prairie has been lost largely due to conversion to row-crop agriculture and non-native grass species for grazing (Samson et al. 2004, Wright and Wimberly 2013, Lark et al. 2015, Wright et al. 2017).

In recent decades, South Dakota, located in the WCB, has experienced some of the highest rates of grassland loss, primarily east of the Missouri river where tallgrass prairies become dominant on the landscape (Wright and Wimberly 2013, Larkin et al. 2015, Wright et al. 2017). This is particularly problematic for a state that receives US$1.3 million annually in spending from fishing, hunting, trapping, wildlife watch, boating, state park visitation, and snowmobiling (Southwick Associates 2017). Ecologically, this high rate of grassland conversion loss is concerning. Within South Dakota, the Coteau des Prairies (referred to as the Prairie Coteau for the rest of this thesis) is characterized by its higher elevation from the surrounding landscape of eastern South Dakota and its location in the Prairie Pothole Region (Figure 2.1-1). The Prairie Coteau is a plateau created during the Wisconsin glaciation period, rising 275 m above the surrounding plains and located primarily in eastern South

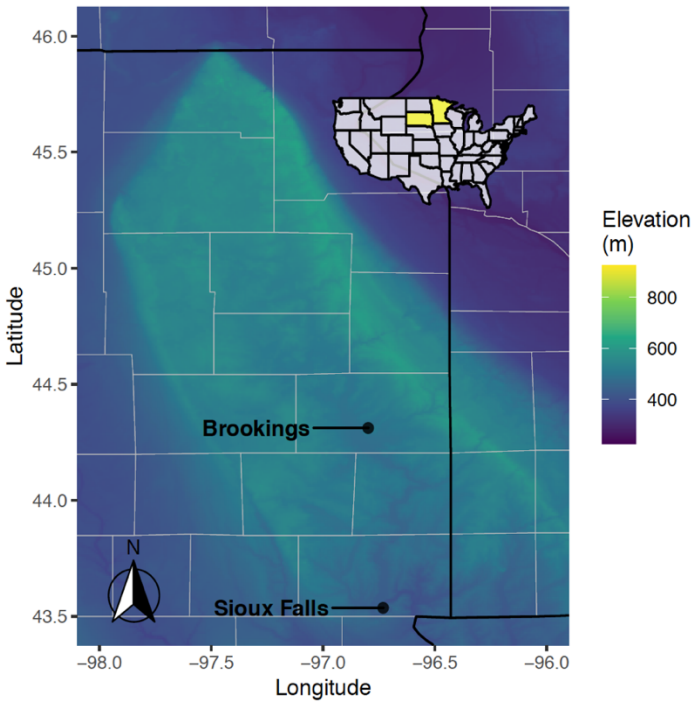

Figure 2.1-1: The Prairie Coteau, located primarily in South Dakota, spans from North Dakota south through South Dakota into Minnesota and Iowa.

Dakota and stretching 200 miles long and 100 miles wide, from southern North Dakota to southwestern Minnesota and northwestern Iowa. Precipitation ranges from 600 mm in the northwest to 800 mm in the southeast. Vegetation on the Prairie Coteau ranges from mixed-grass prairie on the western edges of the Coteau to northern tallgrass prairie on the eastern portion. Land cover is predominantly cropland, with remnant, untilled grassland, non-native seeded hayfield and pasture, and native or non-native retired cropland (such as land in the Conservation Reserve Program) contributing to the matrix of land covers. Ownership is primarily private.

A concerted effort between private and government organizations toward conservation in the region has been underway. Of the remaining grasslands and woodlands on the Prairie Coteau, 39 percent of those are under permanent protection, totaling 3.7 percent of the total Prairie Coteau (Bauman et al. 2014). Much of the conservation work regionally has been focused on the Prairie Pothole Region (PPR) due to its continental importance as breeding grounds for waterfowl

(Batt et al. 1989), and is a major migration route for many other bird species (Skagen et al. 1999, Igl et al. 2017). The PPR extends from North Dakota, South Dakota, Iowa, Minnesota, and Montana in the United States, to Alberta, British Columbia, Manitoba, and Saskatchewan in Canada and is the result of historical glaciation creating thousands of depressions on the landscape, which are then filled by snowmelt or precipitation. Globally, over 85 percent of wetlands have been lost, and the PPR has not been an exception to this trend. The PPR has been experiencing the highest rates of conversion in the region, with one estimate at greater than 16.5 percent loss between 2008 and 2012 (Lark et al. 2015, Wright et al. 2017). Wetlands are critical for many services including water filtration, food supply, and climate regulation and these services are declining due to rapid wetland loss (IPBES 2018).

To manage this region for its long-term health and survival, it is essential that managers can 1) rapidly and correctly identify species and 2) understand how different calculations quantifying an ecosystem can impact management. Traditional methods of species identification usually relies on the presence of mature individuals with characteristic morphology. This requires sufficient training and identification is not always feasible when individuals are damaged or are not in an appropriate stage of development. In addition to problematic and slow species identifications, there are numerous proposed calculations for describing ecosystems and no clear universal metric. It is therefore critical to understand how metric selection can impact conclusions (Humphries et al. 1995, Kenchington and Kenchington 2013, Moreno et al. 2017). We must balance including as much descriptive power as possible, while minimizing costs to ensure it is feasible to employ these measurements.

To address these two questions for tallgrass prairie plant communities occurring on the Prairie Coteau, I developed two projects: 1) examining the utility of DNA barcoding for rapid regional plant identification and 2) comparing biodiversity metrics to inform “best practice” measures for research,

management, and restoration, with a focus on the comparison of multiple phylogenetic diversity metrics with species richness. Although some studies have included species occurring in the region (Braukmann et al. 2017), there have been no community-wide assessments on DNA barcoding, nor have any studies examined the use of phylogenetic diversity and its relationship to species richness in native prairies.

CHAPTER 2: UTILITY OF DNA BARCODING

2.1 ABSTRACT

DNA barcoding, the use of genetic data for identification, has been shown to be useful in many settings. Barcoding has aided in rapid species identification in both plants and animals for conservation, management, and regulation. I examine the success of using four common DNA barcoding regions (ITS, *matK*, *psbA-trnH*, *rbcL*) to identify plants of the tallgrass prairies of the Northern Great Plains using high throughput sequencing. Using species occurring at Oak Lake Field Station in eastern South Dakota, I collected tissue from herbaria collections and amplified the four barcode regions to test success at identifying samples to family, genus, and species using BLAST in a public DNA sequence database. Species level identification ranged from 21% to 66%. Genus and family level identification were generally successful (84-100% and 91-100% respectively) and showed no significant differences. Increasing the number of loci included in a barcode increased success of correct identification, generally. Results indicate the inclusion of ITS and *rbcL* to be beneficial for the identification of regional taxa. Species-level identification was low, however genus and family-level identification showed high success at discrimination, which may be appropriate for applications not needing precise species-level identification.

2.2 INTRODUCTION

Research involving time consuming species identification needs to drastically accelerate to understand the continued loss of imperiled systems (DeSalle and Amato 2004). Many monitoring programs have sacrificed accuracy due to the time it takes to identify plants to species level but in doing so are failing to capture critical information that can be used in further research. For example, as discussed in Love and Cane (2019), prior failure to record detailed herbaceous plant species, and instead grouping plants into the ambiguous category of “forb,” precluded further research, in this case regarding native insect

pollinators. Many field technicians are not fully trained to accurately identify species by sight, and identification using technical dichotomous keys adds additional time to projects. Morphological plasticity in populations, cryptic species, and dependency on life stage are some of the largest issues facing current methods for species identification (Hebert et al. 2003, Hollingsworth et al. 2016). Further compounding the problem is the increasing lack of taxonomists in the field (Drew 2011). So, although morphology is extremely important for identification of species though diagnosable species concepts and in the field, it takes time to do so accurately and requires an expertise that is becoming more scarce (Tewksbury et al. 2014).

DNA barcoding offers one potential solution. DNA barcoding uses small segments of DNA to identify species (Hebert et al. 2003). For example, sequences of the mitochondrial cytochrome oxidase I gene (COI) have been effectively used as a barcode for species-level identification of many animal groups (Hebert et al. 2003), acting as a type of bio-identification system useful for species identification (Handy et al. 2011), invasive species control (Floyd et al. 2010), forensics (Savolainen and Lundeberg 1999), and regulatory enforcement (Parveen et al. 2016), among others. Though DNA barcoding is unlikely to replace field identification of species, it is another tool for when morphological features are not available, whether due to disturbance (*e.g.* grazing or burning), analyzing fecal material, or needing verification for morphological identification. DNA barcoding may circumvent the previously mentioned issues involved in morphological based species identification, as all that is needed is a small amount of tissue, regardless of developmental stage. The advent of high-throughput sequencing (HTS) has opened even more potential to DNA barcodes, as numerous loci and individuals can be sequenced at once, reducing time and cost. Additionally, as costs for HTS continue to decline, there is potential to increase the amount of genetic data retrieved, allowing for increased species identification success (Parks et al. 2009, Nock et al. 2011, Steele and Pires 2011, Kane et al. 2012).

A universal barcode for plants has remained elusive, however. The problem lies with finding a region with high sequence variation between species to create genetic diversity but with low enough variation to allow sequences to be grouped by species, all while being retained across groups. Multiple studies have examined the issue but have yet to find a single gene region able to differentiate taxonomic groups at both small and large scales. The most effective method appears to be a combination of gene regions, such as *matK* and *rbcL* (CBOL Plant Working Group 2009). The CBOL Plant working group found that an average of 72% of tested species can be distinguished using these two regions. Additionally, other gene regions have been proposed to supplement these regions including ITS, *rpoc1, rpoB, psbA-trnH* and *trnL* (Kress et al. 2005, Chase et al. 2007, Hollingsworth et al. 2009, Syme et al. 2013), though they have not been demonstrated to be universally usable across plant groups.

Geographic and taxonomic scales appear to impact success rates of barcodes. For example, Braukmann et al. (2017) examined the utility of barcodes for plant species found across Canadian ecosystems (5108 species) and found success rates when using individual gene regions (*rbcL, matK,* ITS2) ranging from 91-98% in correctly identifying specimens to genus and 44-81% identifying to species. They concluded DNA barcoding "is very effective in identifying Canadian plants to a genus, and that it performs well in discriminating species in regions where floristic diversity is highest." Kress et al. (2009) found success of >98% when identifying woody trees, shrubs, and palms of Panama when using a three-locus barcode (*rbcL, matK,* and *psbA-trnH*). However, studies examining closely related species have resulted in far lower success rates even when using multiple regions (Seberg and Petersen 2009, Spooner 2009).

One ecosystem that could benefit from DNA barcoding is temperate grasslands, one of the most threatened and least protected ecosystems globally (Hoekstra et al. 2005). For sustained use of natural resources in temperate grasslands, species identification is essential. However, identifying plant species

can be difficult due to disturbance (*e.g.* grazing and/or burning) and developmental stage. Additionally, many of the species in tallgrass prairies are closely related and can be difficult to distinguish without a taxonomic key and magnification (*e.g.* species in Poaceae, Cyperaceae, Asteraceae). However, the community composition of tallgrass prairies (highly diverse and closely related clades) could potentially hinder the successful application of DNA barcodes for species identification (Seberg and Petersen 2009, Braukmann et al. 2017).

The use of DNA barcodes holds promise for the identification of these plants regardless of management, disturbance, developmental stage, or cryptic morphology. DNA barcodes have the potential to inform both land managers and researchers on species present in prairie communities to make management decisions and complete research projects where otherwise impossible. This study aims to examine the feasibility of using DNA barcoding to identify tallgrass prairie species. My objectives are to 1) find regions most effective to act as a DNA barcode for identifying tallgrass prairie plant species in the Northern Great Plains and 2) examine feasibility of using DNA barcodes for the identification of regional flora.

2.3 METHODS

2.3.1 SAMPLING

Leaf material was removed from herbarium samples (C.A. Taylor Herbarium [SDC] and Oak Lake Field Station's herbarium at South Dakota State University) based on the Oak Lake Field Station (OLFS) species inventory list (see Supplementary Table 1 and Appendix B for species list and voucher information respectively). This species list consists of 269 species in 63 families, with nearly half of species in Asteraceae, Poaceae, Cyperaceae, and Fabaceae (18, 13, 10, and 8 percent respectively). I prioritized voucher specimens collected at OLFS and as recent of collection as possible. For inventoried species lacking vouchers from the OLFS property, I sampled from herbarium sheets from localities in close proximity

to OLFS. Additional specimens collected at OLFS with species not included in the species list also included. Taxonomic names were updated and standardized using Global Names Resolver (GNR, version 0.9.8) from *taxize* R package version 0.9.92 (Chamberlain and Szocs 2013, Chamberlain et al. 2020).

2.3.2 DNA *EXTRACTION, AMPLIFICATION, AND SEQUENCING*

From each sample, 0.02-0.03 mg of tissue was weighed out for total genomic DNA extraction using a modified 2x CTAB approach (Doyle and Doyle 1987). DNA extractions were then visualized on a gel to assess DNA quality and concentration.

Four loci were selected for this study due to their prominence as "universal" plant barcode regions (ITS, *rbcLa*, *matK*, *psbA-trnH*; Table 2.3-1). *matK* was amplified in two portions to accommodate sequencing read length restrictions in HTS approaches, resulting in five total amplicons being examined as barcodes. These primers were selected due to their ability to amplify across Angiosperm families and produce amplicon lengths compatible with the Illumina (San Diego, California, USA) MiSeq high-throughput sequencing platform limit of 300 base pair (bp) paired-end reads (CBOL Plant Working Group 2009, China Plant BOL Group et al. 2011, Braukmann et al. 2017).

I followed a modified 16S Illumina library construction protocol (Illumina Inc. 2013) with optimized annealing temperatures for each primer set (OligoAnalyzer Tool, Integrated DNA Technologies). This process consists of an initial amplification of target regions by site specific primers ("PCR 1"), with an additional adapter sequence tag added to the 5' end of the oligonucleotide synthesis (sequences obtained from Illumina). These tags allow for the addition of an 8 bp index sequence by acting as a binding site for an additional pair of primers in a second round of PCR ("PCR 2"), which allow for the identification of samples once amplified sequences have been pooled. Library construction used Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific; Waltham, Massachusetts, USA) and the Nexterra XT Index Kit v2 (Illumina). All five

Table 2.3-1: Selected loci and primers for tested DNA barcodes.

Selected Barcodes

Region	*Abbreviation*	*5' primer*	*5' primer sequence from 5' end*	*3' primer*	*3' primer sequence from 5' end*	*Amplicon length*	*References*
ITS2 (nuclear)	ITS	UniPlantF	TGTGAATTGCARRATYCMG	UniplantR	CCCGHYTGAYYTGRGGTCDC	300	(Moorhouse-Gann et al. 2018)
matK (chloroplast)	matK4	matK-1F	ACTGTATCGCACTATGTATCA	matK-4R	GCATCTTTTACCCARTAGCGAAG	400-600	(Bremer et al. 2002)
matK (chloroplast)	matK3	matK-3F	AAGATGCCTCTTCTTTGCAT	matK-3R	GATCCGCTGTGATAATGAGA	400-600	(Sang et al. 1997)
rbcLa (chloroplast)	rbcL	rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC	rbcLa-R	GTAAAATCAAGTCCACCRCG	550	(Kress and Erickson 2007)
psbA-trnH (chloroplast)	trnH	psbA3_f	GTTATGCATGAACGTAATGCTC	trnHf_05	CGCGCATGGTGGATTCACAATCC	500	(Sang et al. 1997)

amplicons for each individual were pooled after PCR1, which gave each individual the same index for identification, while still allowing each region to be identified by primer sequence. Bead cleanup was conducted after PCR2 to remove unwanted reaction components (*e.g.* fragments less than 50 bp) using HighPrep PCR Clean-up System magnetic beads (MAGBIO, Gathersburg, Maryland, USA) in an Apollo 324 System automated library preparation system (IntegenX Inc., Pleasanton, California, USA). All samples were pooled after PCR2 using relative success rates visualized in gel electrophoresis and quantified with QUIBIT 4 Fluorometer (Invitrogen, Waltham, Massachusetts, USA) to standardize concentrations. The pooled library was then sequenced in one run on an Illumina MiSeq platform using 300 bp paired end reads.

2.3.3 READ PROCESSING

Data were received through BaseSpace (cloud-based Illumina software; basespace.Illumina.com) pre-demultiplexed to individual and with index sequences removed. Pooled reads for each individual were run through Fluidigm2PURC (Blischak et al. 2018) on default settings, which trims sequences, combines paired reads, and converts FASTQ file type to fasta format. Because data was pre-demultiplexed by Illumina software, I used a custom script to further group sequences by amplicon and then remove primers (locus_assigner; Fey-Wei Li, Cornell University, personal communication). Consensus sequences were generated using purc_recluster2 (Rothfels et al. 2016) with clustering values of 0.92 and 0.93, and the largest consensus cluster used for downstream analysis. Cleaned sequences were then concatenated in all possible combinations between four single-locus barcodes in Geneious Prime version 2019.2.3 (https://www.geneious.com).

2.3.4 BLAST

Individual loci and concatenated loci (both hereafter referred to as "barcodes") were compared against sequences in the GenBank nucleotide

database, a public nucleotide database of all available sequences (Clark et al. 2016), using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990, Camacho et al. 2009). The top hit for each sequence was used. These results were then compared to original taxon list from OLFS. Family names were generated and species names updated using the Catalogue of Life: 2019 Annual Checklist (http://www.catalogueoflife.org/annual-checklist/2019/) with *taxize* in R for both BLAST results and the original OLFS species list. Success was determined based on family, genus, and species level identification. Results indicating sequencing errors (*e.g.* fungal contaminants, etc.) were removed from analysis.

2.3.5 Analyses

To evaluate identification success, I used *prop.test* in the base R package "stats" (version 3.5.1) to run a proportion test examining if the proportion of identification success for each barcode was equal (alpha of 0.05). *prop.test* uses Pearson's chi-squared test statistic. If significant identification was found, a pairwise proportion test was used (*pairwise.prop.test* in R package "stats") using the Holm (1979) correction method.

2.4 Results

(Barcodes referred to as these abbreviations: ITS2 = ITS, *rbcLa* = *rbcL*, *psbA-trnH* = *trnH, matK*1F4R = *matK4, matk*3F3R = *matK3*)

After updating taxonomic names in *taxize,* I had 266 distinct species for my 286 samples, as some updates combined taxa. *matK3* failed to amplify for most species, with only 22 sequences retrieved (Figure 2.4-1). Due to this low success, *matK3* was excluded from concatenation. *rbcLa* had the highest amplification success with 249 retrieved sequences. Of the 266 taxa at OLFS, 9 species were not represented in GenBank. Additionally, coverage of the tested regions in GenBank varied. 253 of the 266 species had representation for the *rbcLa* region (97%), 246 species were covered by ITS2 (94%), 239 by *matK* (91%), and 123 by *psbA-trnH* (47%). Species not in GenBank were still included in results,

as I was looking for an overall view of barcoding success for regional flora identification. Age of specimen had no correlation with amplification success (mean year collected: 1994, range: 1920-2013; Figure 2.4-2).

Overall, concatenated barcodes had higher success rates than relying on individual loci. *matK4*, *matK3*, and *rbcLa* were the least successful individual loci for species level identification (21%, 29%, and 33% respectively), with *psbA-trnH* fifth worst (39%). ITS2 had the highest success of the single-locus barcodes at 53%. The combination of ITS2, *matk4*, *rbcLa*, and *psbA-trnH* proved to be the most successful at species discrimination, at 66% (Figure 2.4-3; Supplementary Table 2, 3 and 4). When examining the single-locus barcodes, I found significant variation in proportion of correct species-level identification between ITS2 and *rbcLa* and between ITS2 and *matk4*. With the inclusion of concatenated barcodes, I saw additional significant differences in species-level identification success (Figure 2.4-4). Successful genus identification ranged from 84-100% and successful family level identification ranged from 91-100%. There were no significant differences in genus or family-level identification success between barcodes. The number of loci included in the barcode significantly impacted species resolution success but did not impact correct genus or family-level identification success (Supplementary Table 4).

Breaking down the results by the four most diverse families (Asteraceae, Poaceae, Cyperaceae, and Fabaceae with 49, 35, 25, and 22 specimens sampled respectively), I found ITS and *rbcLa* were the most successful in amplification success (Figure 2.4-5). *rbcLa* was most successful in amplification for Asteraceae (30/49), Fabaceae (20/22), and Cyperaceae (23/25), with ITS and *psbA-trnH* performing the best in Poaceae (25/35). The barcode that performed the best at species-level identification varied for each family, with the *rbcLa* and *psbA-trnH* concatenated barcode performing the best in Asteraceae (65%), ITS alone and ITS in combination with *rbcLa* were highest in Fabaceae (69%) and Cyperaceae (43%).

The ITS, *rbcLa*, and *psbA-trnH* concatenated barcode performed the best for Poaceae (35%).

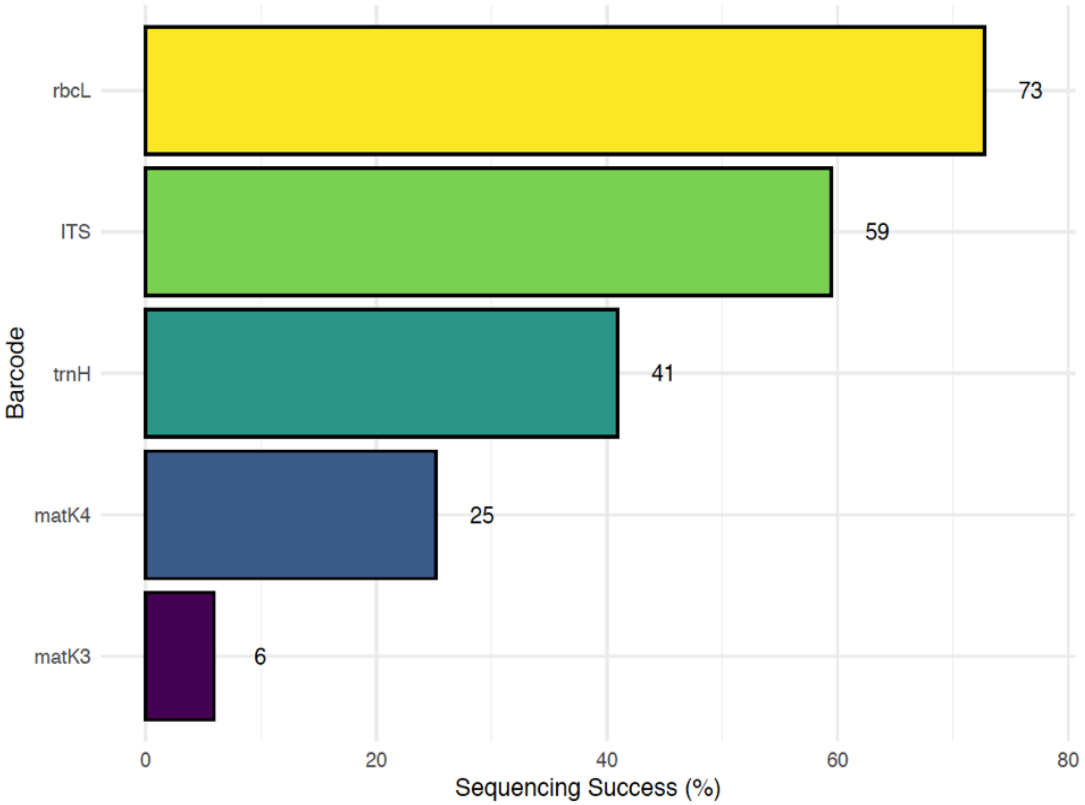

Figure 2.4-1 Sequence retrieval success of amplicons out of 286 specimens sampled.

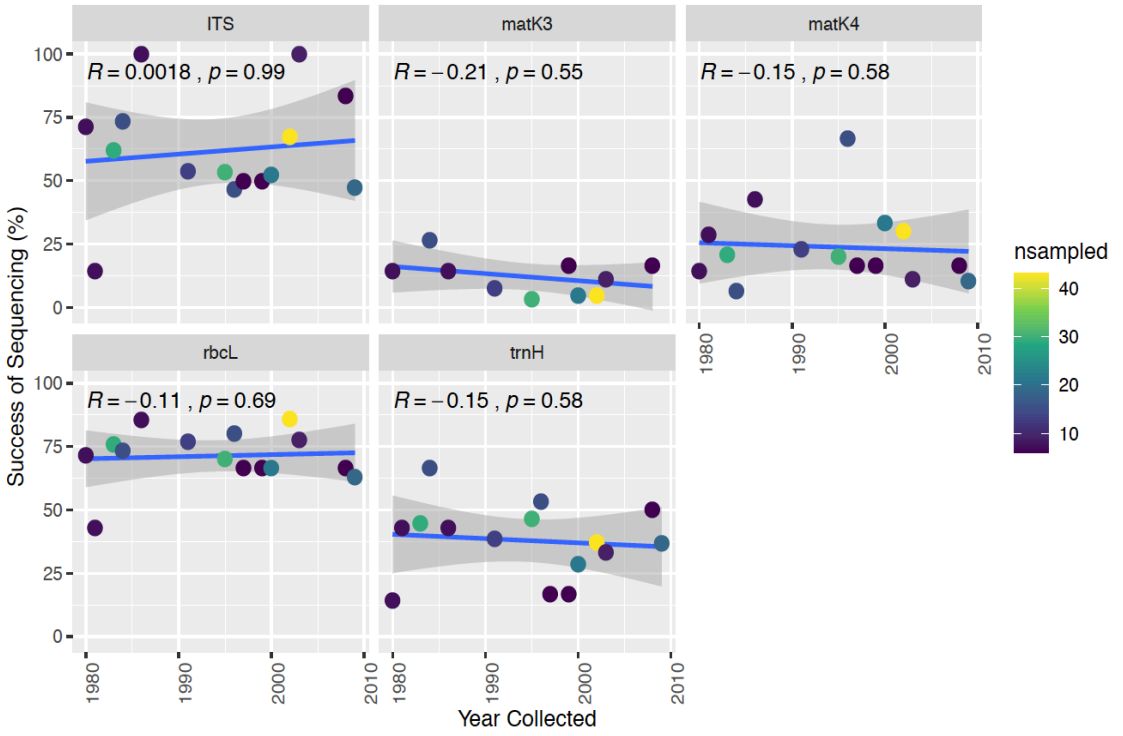

Figure 2.4-2: My success of retrieving amplicons after PCR and sequencing do not appear to be impacted by the year the specimen was collected. Pearson correlation shown.

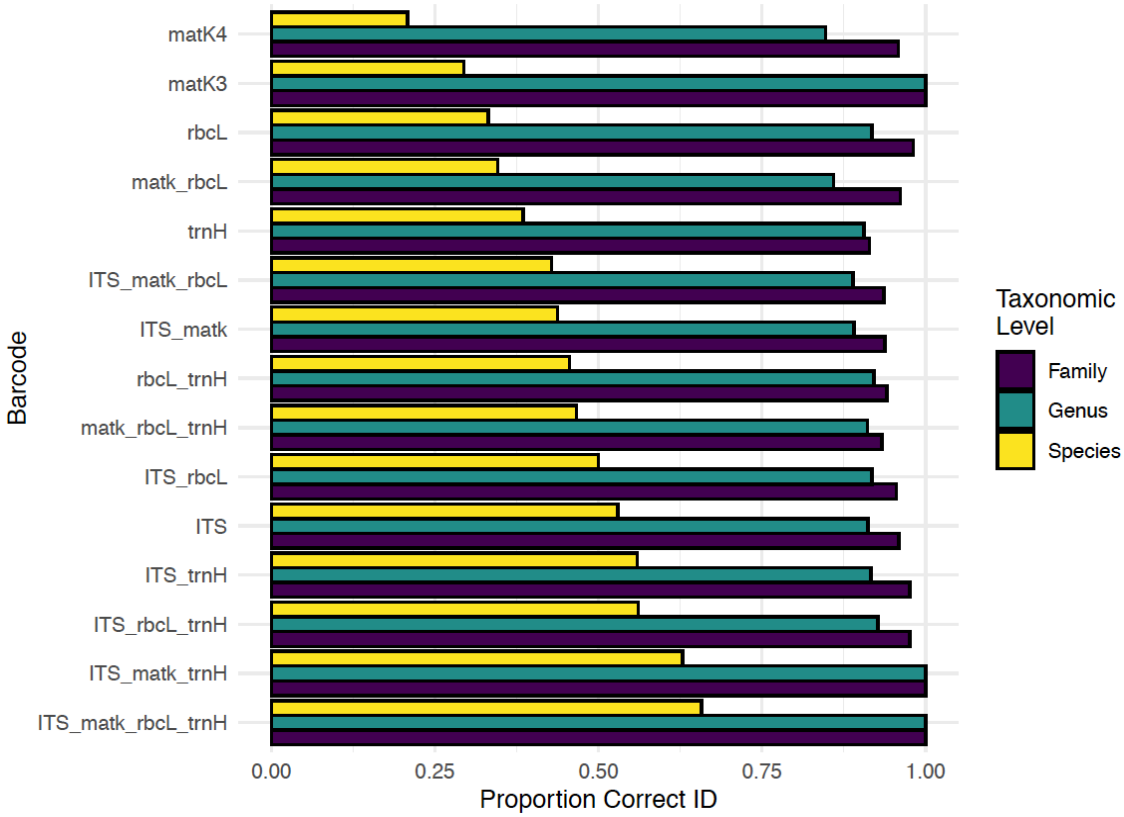

Figure 2.4-3 Success of identification for barcodes. Underscored barcode names indicate concatenated locus barcodes. Note that matK3 was excluded from concatenation.

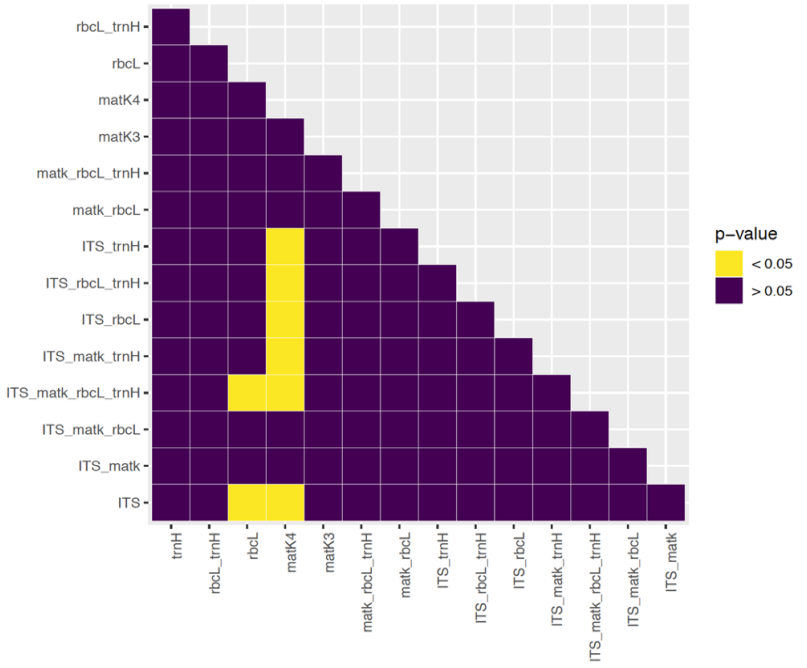

Figure 2.4-4: Pairwise proportion tests indicate significant differences in species identification success.

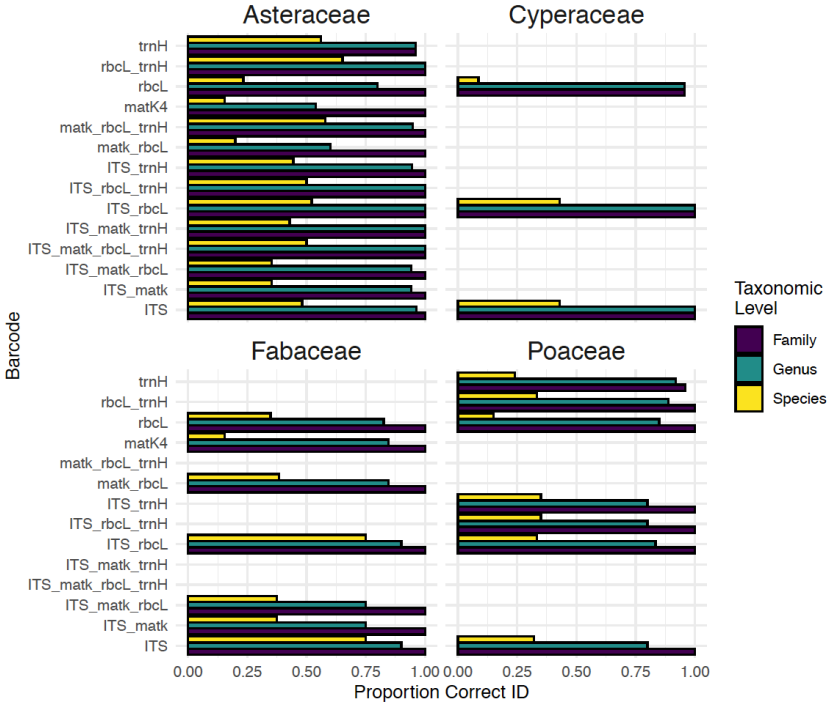

Figure 2.4-5: Barcode success in the four most diverse families. Only barcodes with more than five replicates shown.

2.5 Discussion

My results indicate DNA barcoding is successful at the identification of species at the genus and family taxonomic levels across tested barcodes, but only had low to moderate success at the species level. One-locus barcodes had lower success at species level identification than multi-locus barcodes, in accordance with previous studies. I found significant variation in barcode ability to successfully identify individuals at the species level (21-66% correct identification), with no significant variation in successful identification at the genus or family levels (84-100% and 91-100% respectively). *matK* and *rbcLa* were particularly poor at identifying species when used as a single-locus barcode. Poaceae, one of the families that would likely be of most interest for regional land managers, had low success across examined barcodes.

The low success rate in species discrimination when using *matK* alone contrasts with other studies, where *matK* was one of the most successful barcoding regions for identification in plants (Lahaye et al. 2008, CBOL Plant Working Group 2009, Braukmann et al. 2017). A major limitation in using *matK*, as also noted by previously listed studies, is the difficulty of finding universal primer pairs (Hollingsworth et al. 2011), which is likely why one of my selected barcode regions failed to amplify (*matK3*). In contrast, though *rbcLa* only has moderate identification success, it has been shown to amplify well across taxa, which has led to it being promoted as a good candidate for inclusion in a multi-locus barcode (CBOL Plant Working Group 2009, China Plant BOL Group et al. 2011, Hollingsworth et al. 2011).

The combination of *matK* and *rbcL* has been promoted as one of the most promising universal two-locus plant barcodes (CBOL Plant Working Group 2009). Failure of this two-locus barcode in some systems for species identification, particularly with closely related taxa (Seberg and Petersen 2009, Roy et al. 2010, Parmentier et al. 2013), has resulted in the recommendation of including a nuclear-encoded ribosomal internal transcribed spacer, ITS2 (Chen et al. 2010,

China Plant BOL Group et al. 2011, Hollingsworth et al. 2011). My results correspond with previous studies that the inclusion of ITS is beneficial. The universal presence of ITS2 across plant taxa and its short length (~350) make it a promising barcode for use in community assessments and HTS approaches for DNA barcoding. The high success of sequence recovery and species identification of ITS2 and *rbcLa* in my community, particularly when used jointly, indicate these two regions would be effective for barcoding regional tallgrass prairie plant species.

The poor amplification success of *matK* could be due to several reasons. Increasing age of herbarium specimens has been shown to reduce ability to amplify loci due to the low quality of DNA due to degradation over time (Pyle and Adams 1989, Savolainen et al. 1995, Adams and Sharma 2010, Staats et al. 2011, Sarkinen et al. 2012, Brewer et al. 2019), however my amplification successes and failures do not indicate a trend regarding the date of collection (Figure 2.4-2). The most likely issue is with my chosen primer pairs.

Overall, my results indicate a combination of loci is beneficial, which corresponds with previous studies (Chase et al. 2007, Lahaye et al. 2008, CBOL Plant Working Group 2009, Kress et al. 2009, China Plant BOL Group et al. 2011). Additionally, as costs of HTS continue to decrease, it is more feasible to increase the number of sequenced regions, leading to the proposal of whole plastid genome sequencing for species discrimination (Parks et al. 2009, Nock et al. 2011, Steele and Pires 2011, Kane et al. 2012). For increased success in older herbarium specimens, probe sets such as Angiosperm 353, which targets nuclear single-protein-coding regions, would be beneficial, as the short, conserved regions are well suited for degraded tissue and identification at shallow taxonomic levels (Johnson et al. 2018, Brewer et al. 2019, Larridon et al. 2020).

Additionally, it is important to consider where barcode regions originate, whether nuclear or plastid. Although there are benefits for sequencing the entirety of the chloroplast genome, nuclear regions have the benefit of showing

mixed inheritance. Where chloroplast genomes are inherited maternally, nuclear genes are bi-parentally inherited and can indicate hybridization events (Rieseberg and Soltis 1991, Soltis and Kuzoff 1995). Likely the best methodology for DNA barcoding of regional prairie plant species would be through the creation of a specific probe set suited for this region's plant community, as done for *matK* by Heckenhauer et al. (2016) in their system. This will be especially important for improving the success rates of my most regionally diverse families.

The creation of a regional database (versus large collections such as GenBank), will likely result in increased taxonomic differentiation success. The presence of international species in global database results in more closely related species than are found regionally, lowering species resolution (Parmentier et al. 2013). Additionally, there are multiple species/loci missing from GenBank, so concentrated effort to collect tissue from these species will be of benefit. The creation of a regionally managed database using verified specimens will likely reduce errors.

2.6 Conclusions

My results indicate there is potential for the use of DNA barcoding for tallgrass prairie plant species of the Northern Great Plain, particularly at the family and genus level. Species-level identification rates could be limited, depending on the resolution needed. The optimization of primers for prairie species and the creation of a regional database remain promising future directions that will likely increase in successful identification at these shallow taxonomic scales. I have shown DNA barcoding has potential to identify this regional flora and provides a promising tool to use when morphology-based identification is not feasible in this critically threatened system.

CHAPTER 3: PHYLOGENETIC DIVERSITY

3.1 ABSTRACT

The drastic decline of the once extensive prairies of the Great Plains is of concern, not only for the population declines of many species, but also the decline of ecosystem functionality. Traditional methods of quantifying ecosystems do not include evolutionary history of communities, or phylogenetic diversity (PD), an important feature that can capture genetic diversity and functioning in a community. With costs of sequencing decreasing and availability of prebuilt trees increasing (synthesis phylogenies), it now feasible to cheaply and quickly include measures of evolutionary diversity in management conversations. Twenty-one field sites across the Prairie Coteau of eastern South Dakota were used to examine the relationship between several common PD metrics and species richness of late season tallgrass prairie plant communities. I found little correlation between PD and species richness across sites, and less phylogenetic diversity than expected from the regional pool. This lack of correlation indicates increasing plant species diversity does not result in an expected behavior of PD, which has the potential to impact conservation priorities and management strategies. Fortunately, the low cost and ease of using prebuilt trees makes the inclusion of PD measurements achievable.

3.2 INTRODUCTION

Understanding biodiversity loss hinges upon adequate metrics that capture the presence and role of species. Some metrics, such as species richness (SR), Simpson's diversity index (Simpson 1949), and Shannon's diversity index (Shannon 1948), weigh all species as equally important in a community. Although these metrics are straightforward to calculate, they fail to account for inherent differences in functionality between species in a community. SR has remained one of the dominant metrics due to the ease of calculation (as no additional information is needed than species occurrence) and the ability to compare across

sites (Scott et al. 1987, Myers et al. 2000, Meir et al. 2004, Jenkins et al. 2013, Howard et al. 2020). And although Simpson's index and Shannon's diversity are more informative than SR through their inclusion of community evenness, they do not capture the functioning of the system and species relatedness (Cavender-Bares et al. 2009).

Originally proposed as a proxy measurement for functional diversity, phylogenetic diversity (PD) diversity quantifies the amount, distribution or evenness of evolutionary information contained within a community or groupings of species through measuring the evolutionary distance (*i.e*. branch lengths within a phylogeny) between species. Historically, the inclusion of genetics in conservation would have been inhibited by cost of sequencing and expertise needed to work with sequencing data to generate phylogenetic trees. Where in the early 2000s the cost to sequence one megabase (a million bases) of DNA sequence would run upwards of $10,000, costs have now fallen to roughly $0.01 per megabase (Wetterstrand 2019). With increasing accessibility to DNA sequencing, large databases (*i.e*. GenBank) have amassed significant collections that can be mined to create phylogenies.

Mega-trees based on these data, including those by The Angiosperm Phylogeny Group (2009), Zanne et al. (2014), and Smith and Brown (2018), can be used for the creation of regional synthesis phylogenies. Jantzen et al. (2019) and Li et al. (2019b) found synthesis phylogenies result in PD values strongly correlating with PD values from phylogenies built from gene sequence data (purpose built phylogenies; additionally see Allen et al. (2019)). Similarly, Latvis and Herzog (2019) found PD metrics strongly correlate between synthesis phylogenies and purpose built phylogenies for NGP communities, though there may be an overestimation of PD depending on which backbone topology is used to create the regional synthesis tree (also echoing results in Li et al. 2019b). When deciding which tree to use it is important to understand the underlying assumptions. Where branch lengths of phylograms are indicative of divergence in

features, branch lengths of chronograms indicate divergence in *time*. Interpretations of metrics calculated from either type of tree should reflect this foundational difference. Additionally, some studies have found chronograms to result in increased error versus phylograms (Elliott et al. 2018, Allen et al. 2019, Jantzen et al. 2019).

Multiple forms of PD have been proposed to measure communities (Webb et al. 2002, Cavender-Bares et al. 2009, Cadotte et al. 2010) and examine patterns of diversity or distinctiveness of a set of species, *i.e*. evolutionary distinctiveness (Faith 1992, Isaac et al. 2007, Cadotte and Davies 2010). A benefit to using PD is its promise to capture functional diversity (FD), defined by Tilman (2001) as "the value and range of those species and organismal traits that influence ecosystem functioning", genetic diversity (Cadotte et al. 2012, Moquet et al. 2012, Winter et al. 2013), and evolutionary potential (Forest et al. 2007). The inclusion of PD is beneficial when inventorying communities, as more phylogenetically diverse communities are more productive (Cadotte et al. 2008), stable (Cadotte et al. 2012), diverse at higher trophic levels (Dinnage et al. 2012), and resistant to invasion (Davies et al. 2011, Li et al. 2015). Additionally, PD has a flexibility that can be customized to research priorities. For example, branches on a phylogenetic tree, can be given different weights depending on a variety of factors including, but not limited to, abundance, extinction factor, functionality, and desirability. PD appears to capture more variation in biomass production than taxonomic richness alone, indicating evolutionary relationships capturing trait variation are at least partially responsible for loss of ecosystem functioning when biodiversity decreases (Cardinale et al. 2012). However, some studies have shown PD fails to capture FD as the assumptions of trait conservatism and trait divergence following Brownian motion are violated in some trait groups and clades (Safi et al. 2011, Mazel et al. 2017).

Despite the benefits of using PD and other ecological indicators, SR is pervasively used. Studies have found mixed results on the relationship between

PD and SR. There is evidence between the correlation between SR and PD that would signify maximizing SR would thereby maximize PD (Pérez-Losada et al. 2002, Rodrigues and Gaston 2002). However, there are cases with poor correlation between the metrics that could impact conservation prioritization depending on which metric is used (Forest et al. 2007). The cause for this discrepancy is likely due to the layout of branches (topology) of the regional phylogenetic tree (Rodrigues et al. 2005, Tucker and Cadotte 2013). A phylogeny with anciently diverging branches, with their long terminal branches, would mimic SR, resulting in a high correlation between SR and PD. Conversely, when a tree contains many recently diverging taxa (short terminal branches and long internal branches) SR and PD should share a weaker correlation (Cadotte and Davies 2010). Additionally, when a tree has a few monophyletic groups (common ancestor and all its descendants, or clade) sharing more evolutionary diversity within themselves than other groups, the correlation between SR and PD is weaker (Tucker and Cadotte 2013). An asymmetrical tree such as this would result from regions experiencing unequal rates of extinction or radiation between taxonomic groups (Tucker and Cadotte 2013).

Grasslands are dominated by the diverse and closely related family Poaceae (grasses). This results in a large clade on a phylogenetic tree with a high species diversity but low evolutionary diversity. In the tallgrass prairies of the Northern Great Plains (NGP), Asteraceae, Fabaceae, and Cyperaceae are also species rich (Figure 3.2-1). Communities in the region are thereby dominated by distantly related, large clades, which I hypothesize will likely result in weaker correlation between SR and PD. Few estimates of biodiversity in the region have included evolutionary diversity. Should my predicted disparity between SR and PD hold true, conservation/restoration best management practices would likely be impacted.

With the increasing concern for including evolutionary diversity in conservation considerations (DeSalle and Amato 2004), PD has the potential to

provide more insight into communities than ecological indicators that do not include evolutionary relationships. Understanding how metrics capture a community composition, function, and evolutionary makeup will allow for robust prioritizations in management and conservation. Should SR and PD strongly correlate, it would be a simple task to find and prioritize sites with high SR and thereby maximize the evolutionary diversity. However, if the metrics fail to correlate, decision makers will need to make conscious decisions about how they desire to prioritize sites: low cost species richness or more labor-intensive evolutionary analyses.

This study aims to explore when and why correlation occurs between PD and SR in tallgrass prairies of the Prairie Coteau, a glacial uplift area in the NGP, primarily in South Dakota. With the dominance of a few very diverse but distantly related plant families, correlation between PD and SR were expected to have only weak correlation to each other.

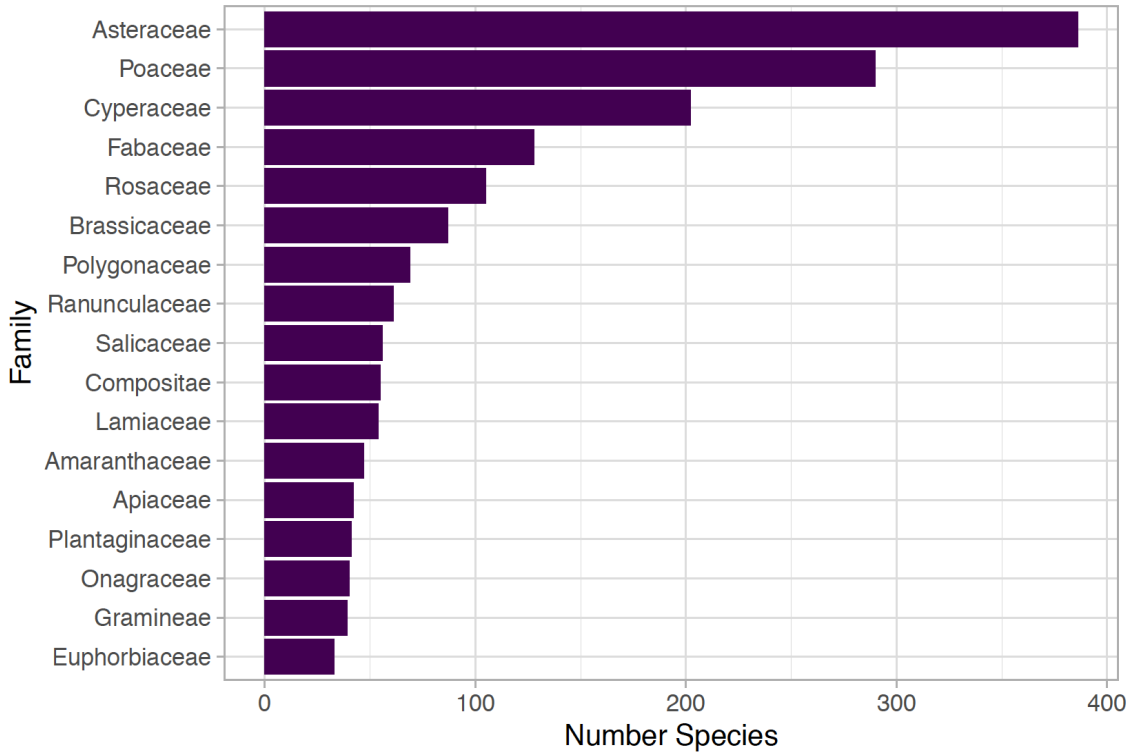

Figure 3.2-1 Only a couple of families are responsible for the majority of diversity in the tallgrass prairies. Here are some of the highest diversity families in the region based on regional herbarium collections in the Consortium of Northern Great Plains Herbaria.

3.3 METHODS

3.3.1 SITES

The Prairie Coteau is a Wisconsin-age glacial moraine, extending from just north of the North Dakota-South Dakota border in Sargent County, ND through 17 counties in South Dakota and 11 counties in Minnesota. Elevation of the Prairie Coteau ranges from 1250 to over 2000 feet above sea level. The high concentration of tracts of conserved land allowed for the selection of tallgrass prairies in a range of conditions and diversity including sites dominated by invasive non-natives, untilled remnants, seeded, grazed and burned sites. In total, 21 transects were placed at 19 sites (two sites were large and had varying management practices employed and were treated as separate samples; Figure 3.3-1 and Supplementary Table 5). Sites were selected based on the expertise of South Dakota Game Fish and Parks, U.S. Fish and Wildlife Services, The Nature Conservancy (TNC) and South Dakota State University (SDState) Extension Office personnel. Landowners included South Dakota Game Fish and Parks, U.S. Fish and Wildlife Services, TNC, and City of Brookings. Owner permission was acquired for each site.

3.3.2 SAMPLING

Sites were visited once between 6 August and 29 August 2019. Sites ranged in size from 20 to 1674 acres. Methods were based off of Barak et al. (2017): two 50 m transects were randomly placed at each site. A random number generator was used to dictate direction of the transect and number of steps from entry point. 0.25 m^2 square quadrats were placed every 5 m, resulting in 10 quadrats placed for each transect. Quadrats were randomly placed 2 to 7 m (1 m increments) away from main transect line on the left or right. Photographs were taken of each transect. Abundances for each species was estimated using six cover classes (Daubenmire 1959).

All species with sufficient populations and in reproductive condition found in quadrats were collected in triplicate and pressed for vouchered herbarium preservation. Taxa were identified, regardless of phenological stage, to the species level using Van Bruggen (1985) with verification from taxonomic experts. Additionally, species in immediate vicinity of transects (~10 m) were collected but were excluded from analysis. All species collected during this study have been digitized and are curated by the C.A. Taylor Herbarium at SDState with records available on the Consortium of Northern Great Plains Herbaria (http://ngpherbaria.org/). Additionally, leaf tissue was preserved in silica gel to create a DNA repository from all possible collected specimens and deposited at SDState.

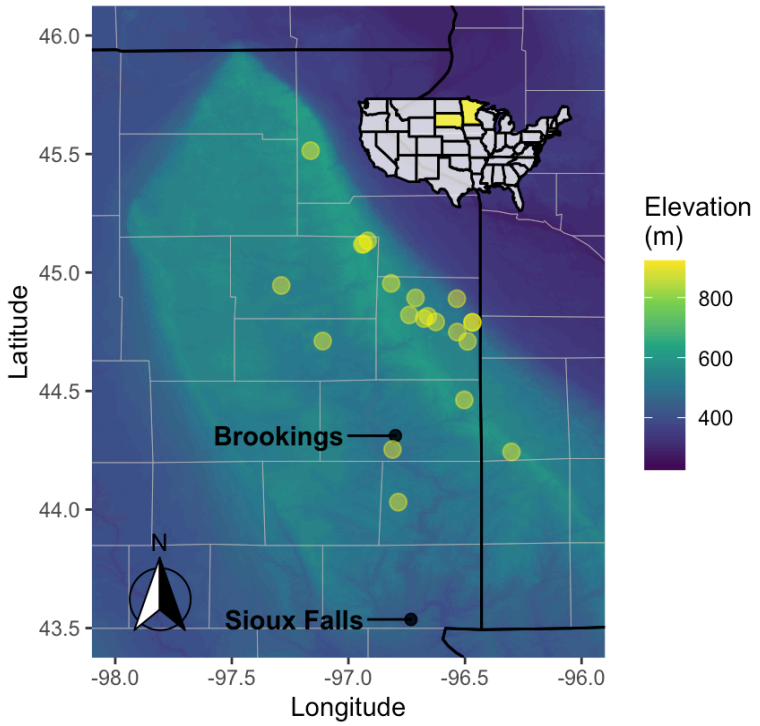

Figure 3.3-1 Field sites were located across the Prairie Coteau. 20 sites were in South Dakota and one site in Minnesota.

3.3.3 PHYLOGENY

To create a phylogenetic tree across all sites (regional tree), I used the R (R Core Team 2018) package V.PhyloMaker version 0.1.0 by Jin and Qian (2019).

V.Phylomaker uses a dated mega-tree derived from Zanne et al. (2014) and Smith and Brown (2018), and includes 74,533 species covering all extant vascular plant families. My tree was constructed using 'scenario 3' to place new genus branches (those genera not in the mega-tree) where tips of a new genus are "bound to the half-way point of the family branch (the branch between the family root node and basal node), unless the family branch is longer than 2/3 of the whole family branch length, in which case the new genus will be bound to the upper 1/3 point of the family branch length." Additional species were bound to the basal node of the genus. For non-monophyletic genera, the most recent common ancestor of the clade with the highest number of branches for genus was used (*build.nodes.1*; see Jin and Qian (2019) for more details). For species found in transects that were identified to the genus level, I used the *add.species.to.genus* function (phytools package in R) to add a "*Genus sp."* branch from the most recent common ancestor node for the genus. In the one instance of a family level identification (Cyperaceae), the observation was removed.

3.3.4 METRICS

Three commonly used phylogenetic diversity metrics were calculated for each site (Table 3.3-1): Faith's phylogenetic diversity (PD_{Faith}), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD). PD_{Faith} measures the sum of all phylogenetic branch lengths at a site (Faith 1992). MPD is the mean pairwise phylogenetic distance between all pairs of taxa (Webb 2000), where MNTD is the mean distance between taxa and their closest relative (Webb et al. 2002). Because MPD summarizes all phylogenetic distances between species in a tree, this metric captures overall phylogeny patterns including deep branching patterns, whereas MNTD captures more recent patterns of closely related species in the terminal branches. Additionally, abundance-weighted metrics were calculated using estimated abundance (percent cover). Species richness (SR) was also calculated for each site.

As SR increases, PD is influenced by the additional branches (Vellend et al. 2011). To remove these effects, standardized effect sizes (SES) were calculated for all three metrics [SES = (observed – expected)/(standard deviation of expected)]. Null (expected) phylogenies were created using 'tip shuffling' and 999 randomizations using *ses.mpd* and *ses.mntd* in the R package picante (version 1.8). This method of randomization shuffles the names of taxa across the phylogenetic tree, resulting in branch length randomization and no modification of the distribution or length. Null models test whether species are being pulled from non-random groups in a phylogeny. SES of PD_{Faith} was calculated using a custom function which maintained 'tip shuffling' randomization (based off code from Swenson (2014)). SES values (referred to as "corrected values" for the rest of this paper) were tested using the p-value (quantile) of observed vs. null communities (observed rank / # runs + 1). Positive values for corrected MPD and MNTD indicate taxa in the community are more distantly related to each other than would be expected by chance (phylogenetic overdispersion), whereas negative values indicate taxa are more closely related to each other than random chance (phylogenetic clustering). Positive corrected PD_{Faith} indicates higher PD than would be expected from a randomly assembled community. Spearman's correlation coefficients were calculated to test relatedness among metrics (alpha=0.05).

Metric	*Definition*	*Calculation*	*R function*	*Citation*
PD_{Faith}	Faith's phylogenetic diversity	Sum of total branch lengths in community.	pd	(Faith 1992)
PD_{SES}	Standardized effect size of PD	Compare observed PD to null communities (expected PD).	ses.pd	(Webb et al. 2008)
MPD	Mean pairwise distance	Average evolutionary distance between all pairwise species; deep tree relatedness.	ses.mpd	(Webb 2000)
MNTD	Mean nearest taxon distance	Average branch lengths connecting each species to its nearest relative; relatedness at tips of tree.	ses.mntd	(Webb et al. 2002)

Table 3.3-1: Calculated phylogenetic diversity metrics using R package picante. Standardized effect scores (SES), or corrected PD, were calculated for Faith's PD, MPD, and MNTD. Abundance weighting was conducted using the option "abundance.weighted = T" in each function, except for weighted Faith's PD, which was calculated using custom code. Output from "ses." functions produce observed PD, expected PD and standardized effect values.

3.4 Results

In total, 928 specimens were collected from the 21 sites representing 47 families and 194 species. SR at each site ranged from 9 to 48 (Figure 3.4-1). The regional phylogenic tree was created from 154 species (representing 39 families and 144 species + 10 identifications to genus level) found in all transects (Figure 3.4-2). Of the 39 families found in the transects, Asteraceae, Poaceae, and Fabaceae were the most diverse (38, 32, and 14 species respectively), representing 58% of the total species pool.

Corrected PD_{Faith} had no significant correlation with SR (R=0.27, p=0.24) (Figure 3.4-3). Corrected MPD and MNTD were not correlated with SR (R=0.19, p=0.41; R=0.43, p=0.052 respectively). Most corrected metric values were negative, though a few were positive, particularly for the unweighted metrics. See Supplementary Table 6 and Supplementary Figure 1 for uncorrected metric values and additional information. Abundance weighting the metrics resulted in no significant correlation with SR for all three PD metrics (R=-0.082, p=0.72; R=0.38, p=0.091; and R=0.11, p=0.63 for PD_{Faith}, MPD, and MNTD respectively).

Multiple communities significantly departed from null communities across the three metrics I evaluated (Figure 3.4-4). When abundance-weighted, more communities deviated from null communities than unweighted communities. Generally, when PD_{Faith} significantly deviated, MPD, and MNTD deviated as well.

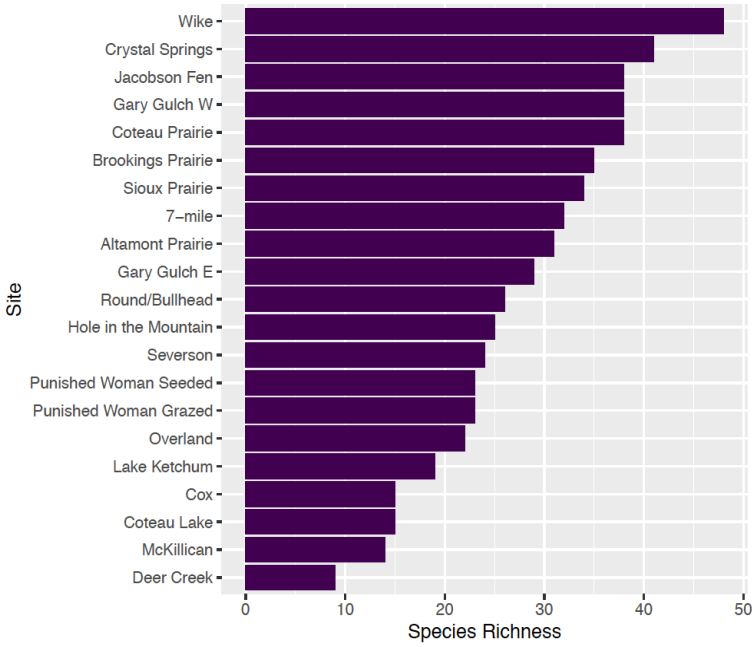

Figure 3.4-2 Species richness varied across sites, ranging from 9 to 48.

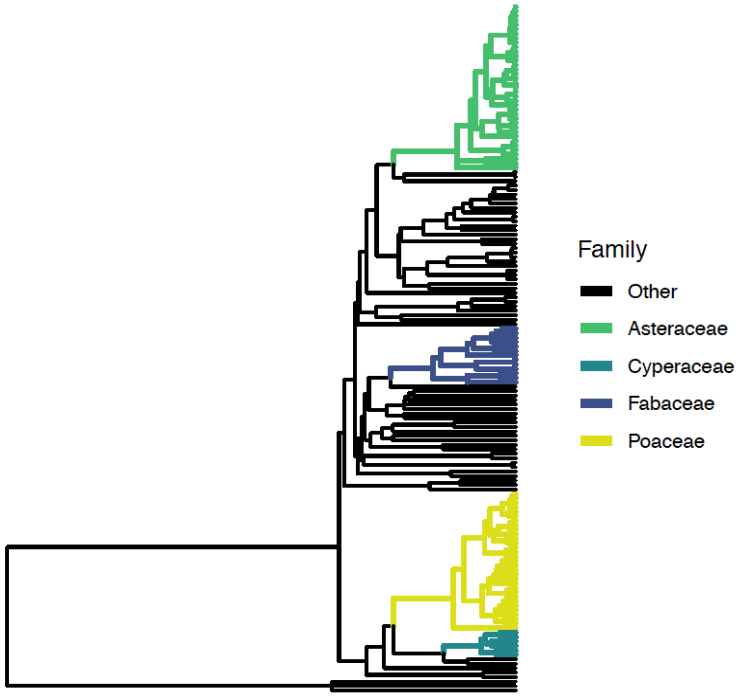

Figure 3.4-1 Regional tree of all species at sampled sites. The four most diverse families have been highlighted, making up over half of all sampled diversity.

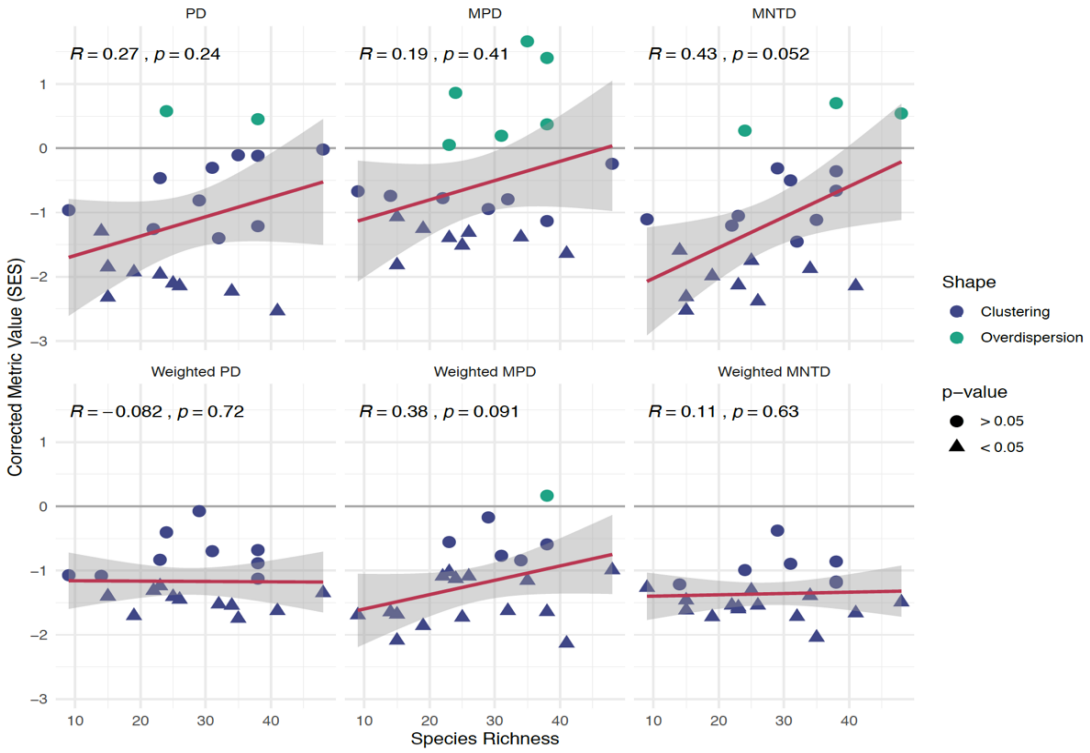

Figure 3.4-3 Correlation between PD, MPD, and MNTD versus species richness for both unweighted (top row) and abundance-weighted (bottom row) calculations. Shape of the tree (clustered or overdispersed) is indicated by point color, and significance is indicated by point shape.

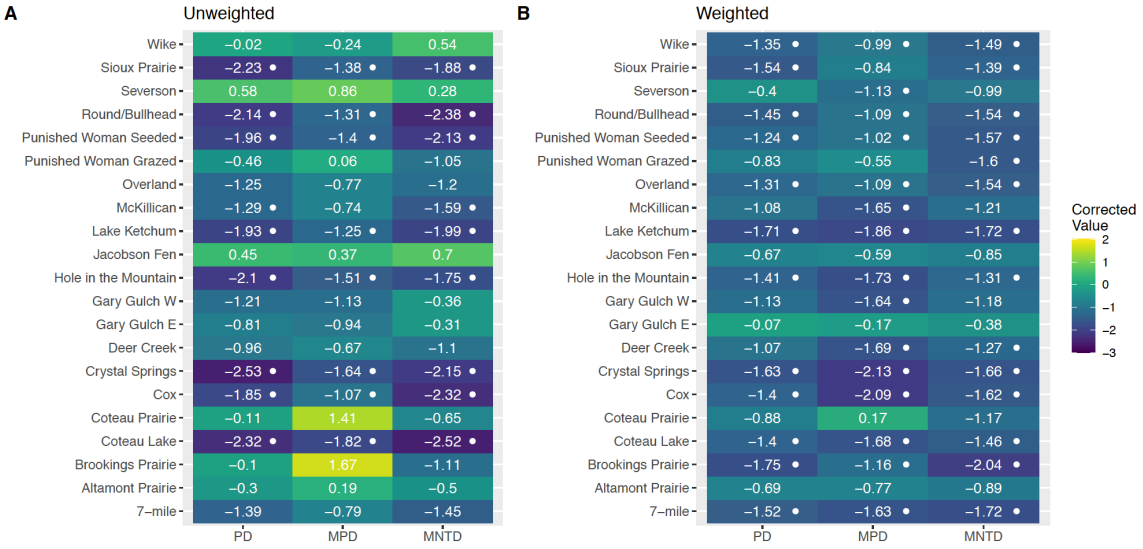

Figure 3.4-4 Corrected PD values for Faith's PD, MPD, and MNTD for both unweighted (A) and abundance-weighted (B) calculations for each site. Color indicates corrected value of PD metric.

3.5 Discussion

My results indicate substantial phylogenetic clustering, or lower PD, in many sites than would be expected from random sampling of the regional tree. I had negative values for almost all metrics at all three levels measured (PD_{Faith} = full tree, MPD = branch behavior across tree, MNTD = terminal branch behavior). When my three metrics were weighted by species abundance, I saw an increased level of clustering. This is unsurprising, as the most abundant species were predominantly those occurring in the species rich clades (*i.e.* Poaceae, Asteraceae, and Fabaceae). Clustering occurs when communities are filtered (*i.e.* due to high levels of disturbance, seasonality, limited precipitation, *etc*.), resulting in the success of closely related species, versus unfiltered communities not experiencing these stressors tending to exhibit over-dispersion (more distantly related taxa) due to increased competition in closely related species (Dayton 1971, Grime 1973, Connell and Slatyer 1977, Webb 2000, Dinnage 2009). Clustering of my sampled communities is unsurprising, as temperate prairies (due to their limited water, reliance on disturbance, and seasonality) are likely historically phylogenetically clustered and, with increased anthropogenic disturbance and climate change, likely to become increasingly less phylogenetically diverse (Wiens and Donoghue 2004, Kerkhoff et al. 2014, Larkin et al. 2015, Li et al. 2019a, Zhu et al. 2019).

My results also indicate no strong correlation between PD and SR when looking across all sites. Thus, managers/biologists cannot increase species richness and gain an expected increase in PD. This corresponds with previous studies that have found a disconnect between historical conservation prioritization that focused on protecting the greatest number of species, which may not correspond with the most evolutionarily diverse sites (Forest et al. 2007, Brum et al. 2017, Pollock et al. 2017, Rosauer et al. 2017, Daru et al. 2019). My results indicate that future studies are strongly advised to take further action to calculate PD metrics, rather than relying on species richness alone. The inclusion

of PD is beneficial when inventorying communities, as more phylogenetically diverse communities are more productive (Cadotte et al. 2008), stable (Cadotte et al. 2012), diverse at higher trophic levels (Dinnage et al. 2012), and resistant to invasion (Davies et al. 2011, Li et al. 2015).

This study was conducted as a snapshot of the Prairie Coteau's tallgrass prairies in August 2019, capturing predominantly warm-season species present during this time of year. Additionally, 2019 experienced one of the wettest years on record for the region (NWS Sioux Falls NOAA), which may influence some species presence and abundance results. My inclusion of Equisetaceae when sampling resulted in the addition of large branch lengths to my trees; inclusion of these distantly related taxa (and other non-flowering clades) could impact conclusions if they are not consistently added or excluded from measurements.

Sampling at one time of the growing season has the potential to influence observed PD by undersampling taxonomic diversity (Park et al. 2018, Jantzen et al. 2019) due to temporal phenological niche separation and phenological conservatism (*i.e*. flowering time) (Kochmer and Handel 1986, Wright and Calderon 1995, Davies et al. 2013). However, my sampling methods included all living individuals, regardless of phenology, reducing potential sampling effects. In comparison to a regional phylogeny I created from regional species documented in the Consortium of Northern Great Plains Herbaria, my August sampling covers the breadth of regional taxa (Figure 3.5-1). However, there are many regionally rare clades that were not captured in my sampling that could add significant evolutionary history, which could be due to seasonality or my restricted sampling area. Increasing the area covered by transects would result in increased taxon coverage and include more regionally rare species. Examining occurrence data from the Consortium of NGP Herbaria, August sampling captures the majority of regional plant families indicating PD results may not be greatly impacted by reduced sampling (Supplementary Figure 2). The PD effects of seasonally biased

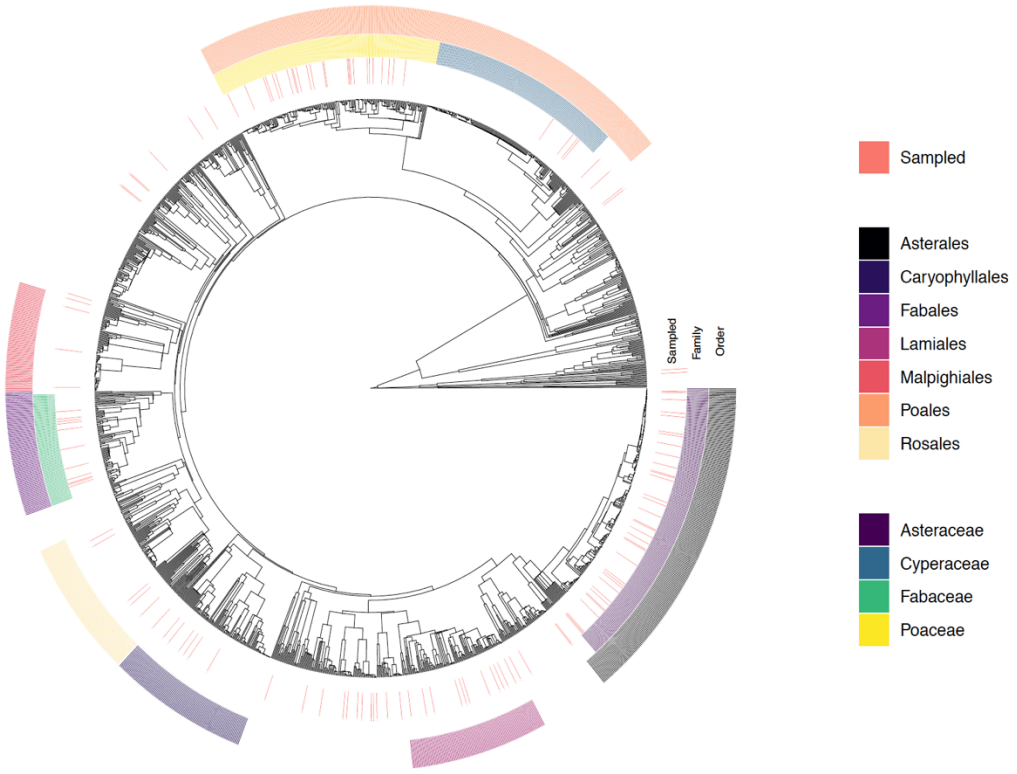

Figure 3.5-1: My August sampled species in relation to all species occurrence records for the region (data collected from the Consortium of the Northern Great Plains Herbaria) covers the breadth of regional taxonomic diversity. The first ring outside of the tree indicates species that have been sampled (red dash = sampled). The next ring out indicates the four most diverse families. The outermost ring shows the seven most diverse orders.

sampling were outside the scope of this project, but would be beneficial for future study to optimize sampling techniques.

My results, in addition to other recent studies, indicate projects need not incur the high costs associated with sequencing genetic information, nor need the expertise to create phylogenies from sequences and can instead rely on prebuilt trees to calculate common PD metrics (Allen et al. 2019, Jantzen et al. 2019, Latvis and Herzog 2019, Li et al. 2019b). There are some important considerations when creating these trees. Care should be taken when making trees that the conclusions match the input data. Choice of tree type, phylogram or chronogram, and which taxa are included (*e.g.* just angiosperm or all plant species) is critical when interpreting results to ensure accurate conclusions.

Future work regarding the relationships between PD and FD (functional diversity) in the tallgrass prairies of the Northern Great Plains will be informative

for multiple fields. Early studies of PD supported the idea that more closely related species will share a high degree of functional diversity (Webb et al. 2002, Cadotte et al. 2011, Flynn et al. 2011). Though this has been found in some cases, there are multiple cases where this assumption has not held true (Mouquet et al. 2012, Mazel et al. 2017). Even if the original promise of PD as a proxy for capturing FD may not hold in all cases, it may still be wise to use it as a bet-hedging strategy (Forest et al. 2007). Using PD to inform restoration efforts can be of benefit as well, as utilizing PD's potential to capture ecosystem function can inform restoration (Verdú et al. 2012, Barak et al. 2017, Barber et al. 2017). Understanding how grazing, a dominant use of land globally, impacts plant communities can help guide management to preserve the benefits of highly diverse plant communities (Larkin et al. 2015, Zhu et al. 2019).

3.6 Conclusions

Multiple studies have found the addition of evolutionary history to be important when trying to understand ecosystem health and functionality (Frankham 2010, Brodersen and Seehausen 2014, Di Marco 2019). Barriers of cost and computation for DNA sequencing have been greatly reduced in recent years, allowing for their increased inclusion into the conservation. My results that PD and SR do not correlate indicate the pervasive use of maximizing SR is likely not maximizing PD and the benefits it can provide for ecosystems. With the drastic decrease in the once widespread tallgrass prairies of North America, it is critical we can understand communities to make informed best management decisions.

Overall Conclusions

Biodiversity is being lost at an alarming rate globally. Population declines, extinctions, and loss of ecosystem functionality will have major implications for future community assemblages. The prairies of the Northern Great Plains are one such system that has been greatly reduced, now occupying less than half of its historical range and experiencing continued conversion. Likely one of the most impacted systems in the Northern Great Plains are the tallgrass prairies, which have been reduced to less than one percent of their historical extent. There has been much effort in the Prairie Coteau region of South Dakota to conserve and protect the plant and animal populations for long-term survival. To make best management practices, decision makers need to 1) be able to identify species rapidly and accurately and 2) know how to best quantify communities. My two projects aimed to address these through 1) DNA barcoding for the identification of regional plant species and 2) exploring the relationship between species richness and phylogenetic diversity.

The results of my DNA barcoding study indicate we are able to successfully identify species to genus and family level at high rates, but identification to the species level is likely not sufficient. ITS and *rbcL* are beneficial when included in a multi-locus barcode. Additionally, the inclusion of more than one loci increases identification success. Future work should examine the benefits to creating a regional database and utilizing high throughput sequencing to increase the genetic coverage to potentially increase identification success.

I found little correlation between PD and SR for regional tallgrass prairie plant communities. If managers desire to incorporate the benefits from increasing PD, increasing SR will not result in predictable PD results. Additionally, many of the sites sampled were less phylogenetically diverse than expected, indicating there is potential benefit to managing these areas to increase evolutionary diversity. Fortunately, the inclusion of PD is not onerous, as mega-phylogenies can

be used and already developed programs make the creation of a regional phylogeny straightforward for non-experts.

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APPENDIX A

Supplementary Table 1: Oak Lake Field Station species list. Primarily compiled by Dr. Gary Larson (retired).

Scientific Name
Achillea millefolium
Aesculus glabra
Agoseris glauca
Agrimonia striata
Agrostis stolonifera
Allium stellatum
Ambrosia artemisiifolia
Ambrosia psilostachya
Amelanchier alnifolia
Amorpha canescens
Amorpha fruticosa
Amphicarpaea bracteata
Andropogon gerardii
Anemone canadensis
Anemone cylindrica
Anemone patens
Apocynum cannabinum
Arctium minus
Artemisia frigida
Artemisia ludoviciana
Asclepias incarnata
Asclepias speciosa
Asclepias syriaca
Asclepias verticillata
Asclepias viridiflora
Aster ericoides
Aster lanceolatus
Aster nova-angliae
Aster oblongifolius
Aster sericeus
Astragalus crassicarpus
Bidens frondosa
Bouteloua curtipendula
Bromus inermis
Calamagrostis canadensis
Calamagrostis stricta
Caltha palustris
Calylophus serrulatus
Calystegia macounii
Calystegia sepium
Capsella bursa-pastoris
Caragana arborescens
Cardamine bulbosa
Carduus nutans
Carex aquatilis
Carex blanda
Carex brevior
Carex cristatella
Carex emoryi
Carex granularis
Carex gravida
Carex hystericina
Carex meadii
Carex molesta
Carex pellita
Carex sartwellii

Carex sprengelii
Carex stricta
Carex tenera
Carex tetanica
Carex utriculata
Carex vulpinoidea
Celastrus scandens
Celtis occidentalis
Ceratophyllum demersum
Cicuta bulbifera
Cicuta maculata
Cirsium arvense
Cirsium flodmanii
Cirsium vulgare
Comandra umbellata
Conyza canadensis
Cornus sericea
Crataegus succulenta
Cryptotaenia canadensis
Cypripedium candidum
Dalea purpurea
Delphinium virescens
Dicentra cucullaria
Dichanthelium acuminatum
Dichanthelium oligosanthes var. scribnerianum
Dichanthelium wilcoxianum
Echinacea angustifolia
Elaeagnus angustifolia
Eleocharis erythropoda
Eleocharis palustrus
Elymus repens
Elymus villosus
Elymus virginicus
Epilobium leptophyllum
Equisetum arvense
Equisetum laevigatum
Erigeron philadelphicus
Erigeron strigosus
Eriophorum angustifolium
Erysimum cheiranthoides
Eupatorium maculatum
Eupatorium perfoliatum
Eupatorium rugosum
Euthamia graminifolia
Festuca subverticillata
Fragaria virginiana
Fraxinus pennsylvanica
Galium aparine
Galium boreale
Galium trifidum
Galium triflorum
Gentiana puberulenta
Geum aleppicum
Geum canadense
Geum triflorum
Gleditsia triacanthos
Glyceria grandis
Glyceria striata
Glycyrrhiza lepidota
Grindelia squarrosa
Helenium autumnale
Helianthus grosseserratus
Helianthus maximiliani
Helianthus nuttallii
Helianthus pauciflorus
Heliopsis helianthoides

Hierochloe hirta
Hordeum jubatum
Hydrophyllum virginianum
Impatiens capensis
Juncus dudleyi
Juncus nodosus
Juniperus virginiana
Koeleria macrantha
Kuhnia eupatorioides
Lactuca canadensis
Lathyrus palustris
Lathyrus venosus
Lemna trisulca
Lemna turionifera
Lepidium densiflorum
Liatris aspera
Liatris punctata
Liparis loeselii
Lithospermum canescens
Lithospermum incisum
Lobelia spicata
Lonicera tatarica
Lycium barbarum
Lycopus americanus
Lycopus asper
Lycopus uniflorus
Lygodesmia juncea
Lysimachia ciliata
Lysimachia thyrsiflora
Matricaria matricarioides
Medicago lupulina
Medicago sativa
Melilotus albus
Melilotus officinalis
Mimulus glabratus
Mirabilis nyctaginea
Monarda fistulosa
Muhlenbergia cuspidata
Oenothera biennis
Onosmodium molle
Osmorhiza longistylis
Oxalis stricta
Oxalis violacea
Panicum virgatum
Parthenocissus vitacea
Pedicularis lanceolata
Penstemon albidus
Phalaris arundinacea
Phleum pratense
Phlox pilosa
Phryma leptostachya
Physalis virginiana
Picea abies
Picea glauca
Pinus ponderosa
Plantago major
Platanthera hyperborea
Poa palustris
Poa pratensis
Polygonatum biflorum
Polygonum amphibium
Polygonum aviculare
Polygonum coccineum
Populus deltoides
Populus X jackii
Potentilla anserina

Prunus americana
Prunus virginiana
Psoralea argophylla
Psoralea esculenta
Pyrus baccata
Quercus macrocarpa
Ranunculus hispidus
Ratibida columnifera
Rhamnus cathartica
Rhus glabra
Ribes americanum
Ribes missouriense
Rorippa palustris subsp. fernaldiana
Rosa arkansana
Rubus occidentalis
Rudbeckia laciniata
Rumex crispus
Rumex orbiculatus
Salix alba
Salix amygdaloides
Salix exigua
Sambucus canadensis
Schizachyrium scoparium
Scirpus microcarpus
Scirpus pallidus
Scirpus tabernaemontani
Scrophularia lanceolata
Scutellaria lateriflora
Senecio plattensis
Senecio pseudaureus
Setaria glauca
Setaria viridis
Shepherdia argentea
Silphium perfoliatum
Sisyrinchium campestre
Smilacina stellata
Solidago canadensis
Solidago gigantea
Solidago missouriensis
Solidago rigida
Sorghastrum nutans
Sparganium eurycarpum
Spartina pectinata
Sphenopholis obtusata
Sporobolus compositus
Sporobolus heterolepis
Stachys palustris
Stellaria longifolia
Stellaria media
Stipa spartea
Stipa viridula
Symphoricarpos occidentalis
Taraxacum officinale
Thalictrum dasycarpum
Toxicodendron rydbergii
Tradescantia bracteata
Tragopogon dubius
Trifolium pratense
Trifolium repens
Typha angustifolia
Typha latifolia
Typha X glauca
Ulmus americana
Ulmus pumila
Urtica dioica
Verbena bracteata

Verbena hastata
Verbena stricta
Vernonia fasciculata
Veronica anagallis-aquatica
Veronica peregrina
Vicia americana
Viola canadensis
Viola nephrophylla
Viola pedatifida
Viola sororia
Vitis riparia
Zigadenus elegans
Zizia aptera
Zizia aurea

Supplementary Table 2: Impact of increasing number of loci in barcode on successful identification at the species, genus, and family levels. Number of sequences and proportions given.

Number of Loci

Loci	*Sequences*	*Family*	*Genus*	*Species*	*PropFamily*	*PropGenus*	*PropSpecies*
1	584	560	530	224	0.96	0.91	0.38
2	485	463	439	227	0.95	0.91	0.47
3	225	216	208	116	0.96	0.92	0.52
4	35	35	35	23	1.00	1.00	0.66

Supplementary Table 3: Identification success for tested barcodes (individual and concatenated loci). Concatenated loci barcodes indicated by inderscore. Number of sequences and proportion success provided. Sequencing success is the ability to amplify and retrieve sequences and is reported in percent.

Barcode Success

Locus	*Sequences*	*Family*	*Genus*	*Species*	*PropFamily*	*PropGenus*	*PropSpecies*	*SequecingSuccess*
ITS	170	163	155	90	0.96	0.91	0.53	59.44
ITS_matk	64	60	57	28	0.94	0.89	0.44	22.38
ITS_matk_rbcL	63	59	56	27	0.94	0.89	0.43	22.03
ITS_matk_rbcL_trnH	35	35	35	23	1.00	1.00	0.66	12.24
ITS_matk_trnH	35	35	35	22	1.00	1.00	0.63	12.24
ITS_rbcL	158	151	145	79	0.96	0.92	0.50	55.24
ITS_rbcL_trnH	82	80	76	46	0.98	0.93	0.56	28.67
ITS_trnH	84	82	77	47	0.98	0.92	0.56	29.37
matk_rbcL	78	75	67	27	0.96	0.86	0.35	27.27
matk_rbcL_trnH	45	42	41	21	0.93	0.91	0.47	15.73
matK3	17	17	17	5	1.00	1.00	0.29	5.94
matK4	72	69	61	15	0.96	0.85	0.21	25.17
rbcL	208	204	191	69	0.98	0.92	0.33	72.73
rbcL_trnH	101	95	93	46	0.94	0.92	0.46	35.31
trnH	117	107	106	45	0.91	0.91	0.38	40.91

Supplementary Table 4: p-values from R function *prop.test* when testing identification success rates between all barcodes and the number of loci included in barcodes at three taxonomic levels: family, genus, and species.

Test	*Level*	*p-value*
All Barcodes	Species	8.95E-08
	Genus	0.299
	Family	0.288
Number of Loci	Species	1.11E-04
	Genus	0.237
	Family	0.633

Supplementary Table 5: Plant communities sampled. TNC = The Nature Conservancy.

Sites

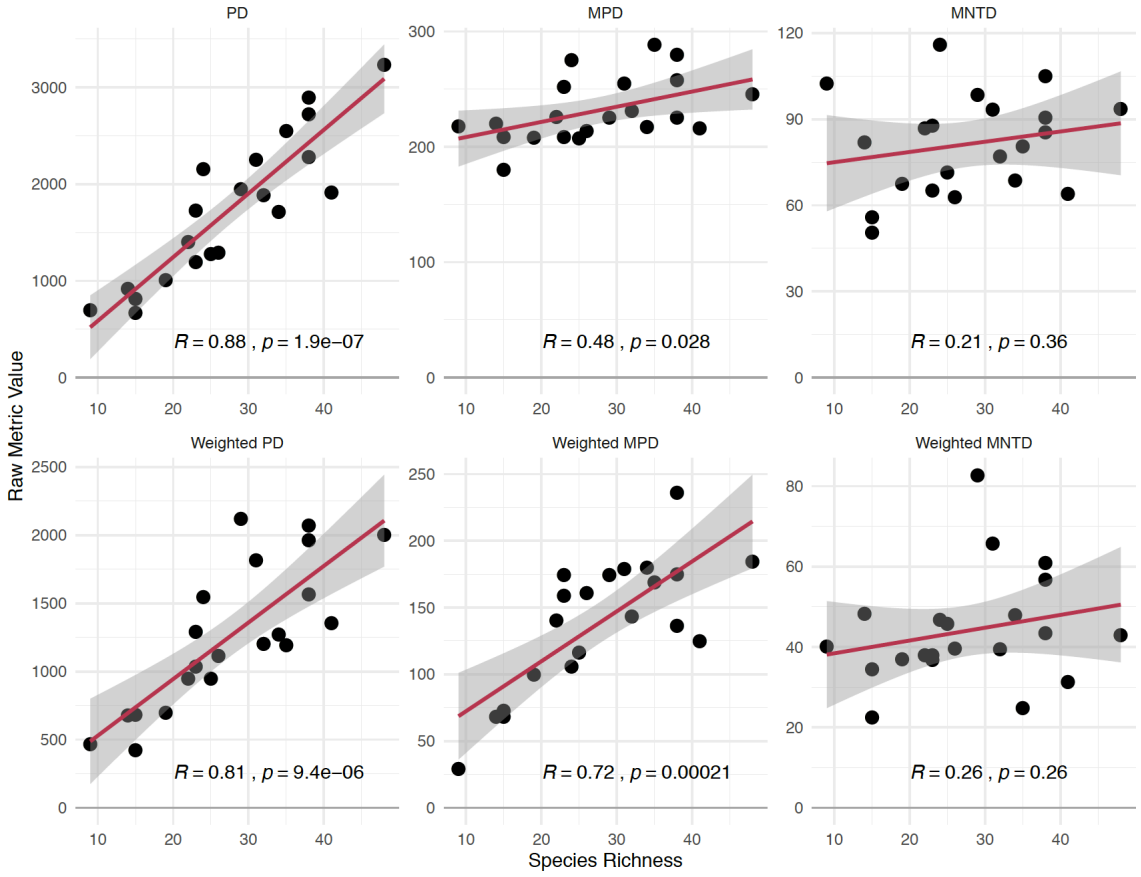
Site	*Latitude*	*Longitude*	*County*	*State*	*Elevation (m)*	*Date Sampled*	*Owner*
7-mile	44.75	-96.53	Deuel	South Dakota	504	7-Aug-19	TNC
Altamont Prairie	44.89	-96.53	Deuel	South Dakota	443	9-Aug-19	TNC
Brookings Prairie	44.25	-96.81	Brookings	South Dakota	483	29-Aug-19	City of Brookings
Coteau Lake	44.82	-96.74	Deuel	South Dakota	563	11-Aug-19	State
Coteau Prairie	44.89	-96.71	Deuel	South Dakota	545	13-Aug-19	Federal
Cox	44.71	-97.11	Hamlin	South Dakota	535	21-Aug-19	Federal
Crystal Springs	44.82	-96.66	Deuel	South Dakota	574	9-Aug-19	State
Deer Creek	44.46	-96.50	Brookings	South Dakota	535	27-Aug-19	State
Gary Gulch E	44.79	-96.47	Deuel	South Dakota	474	14-Aug-19	State
Gary Gulch W	44.79	-96.47	Deuel	South Dakota	474	8-Aug-19	State
Hole in the Mountain	44.24	-96.30	Lincoln	Minnesota	565	22-Aug-19	TNC
Jacobson Fen	44.79	-96.63	Deuel	South Dakota	529	7-Aug-19	TNC
Lake Ketchum	44.81	-96.68	Deuel	South Dakota	538	12-Aug-19	State
McKillican	44.95	-97.29	Codington	South Dakota	535	15-Aug-19	State
Overland	45.13	-96.92	Codington	South Dakota	580	19-Aug-19	Federal
Punished Woman Grazed	45.12	-96.94	Codington	South Dakota	575	16-Aug-19	State
Punished Woman Seeded	45.12	-96.93	Codington	South Dakota	569	19-Aug-19	State
Round/Bullhead	44.95	-96.82	Deuel	South Dakota	575	13-Aug-19	State
Severson	44.71	-96.49	Deuel	South Dakota	525	6-Aug-19	Federal
Sioux Prairie	44.03	-96.79	Moody	South Dakota	517	23-Aug-19	TNC
Wike	45.51	-97.16	Roberts	South Dakota	599	20-Aug-19	Federal

Supplementary Table 6: Species richness, Faith's PD, MPD, and MNTD values for observed (obs.) and null (rand.) communities. Values include: pd, mean, standard deviation (.sd), rank, standardized effect score (.z), and p-value (.p) for abundance-weighted (wt.) and unweighted metrics.

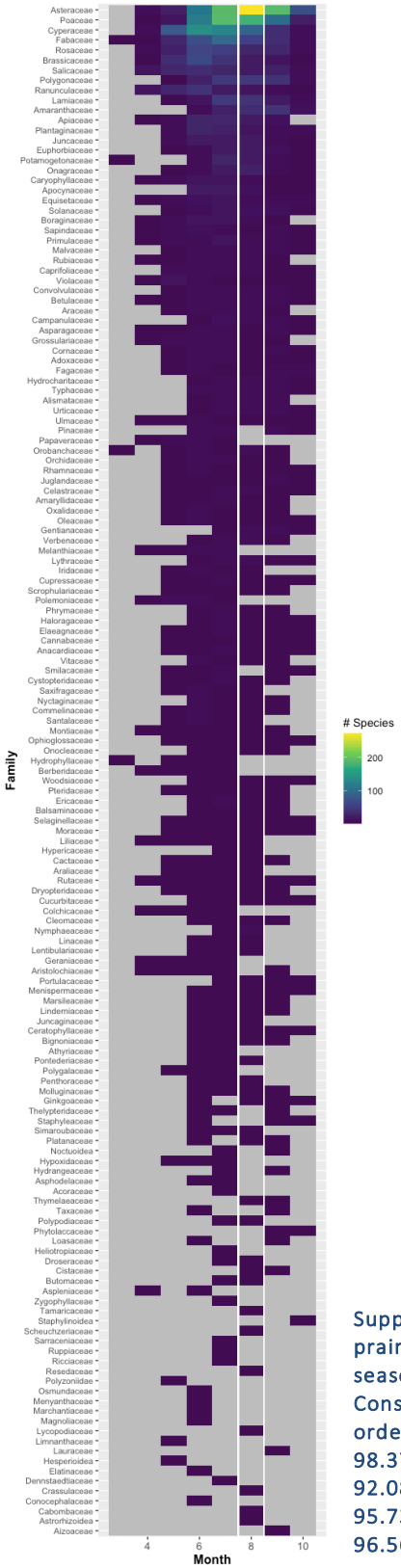
site	*Gary Gulch E*	*Deer Creek*	*Crystal Springs*	*Cox*	*Coteau Prairie*	*Coteau Lake*	*Brookings Prairie*	*Altamont Prairie*	*7-mile*
ntaxa	29	9	41	15	38	15	35	31	32
pd.obs	1953.07	698.34	1918.21	815.09	2726.20	670.98	2550.67	2254.14	1888.24
pd.rand.mean	2244.83	942.40	2882.37	1382.94	2767.41	1374.21	2590.82	2368.69	2426.96
pd.rand.sd	362.06	255.14	380.47	306.88	376.80	302.89	383.21	381.57	386.62
pd.obs.rank	278	112	3	4	414	1	437	414	92
pd.obs.z	-0.81	-0.96	-2.53	-1.85	-0.11	-2.32	-0.10	-0.30	-1.39
pd.obs.p	0.28	0.11	0.00	0.00	0.41	0.00	0.44	0.41	0.09
wt.pd.obs	2121.57	469.12	1355.43	682.74	2071.87	424.41	1196.58	1816.16	1206.02
wt.pd.rand.mean	2194.23	928.85	2838.91	1342.00	2656.55	1401.33	2523.96	2311.81	2380.87
wt.pd.obs.z	-0.07	-1.07	-1.63	-1.40	-0.88	-1.40	-1.75	-0.69	-1.52
wt.pd.obs.p	0.54	0.07	0.01	0.03	0.17	0.00	0.00	0.25	0.01
wt.pd.rand.sd	992.61	430.74	911.50	470.35	661.15	696.76	759.54	717.34	770.69
mpd.obs	225.59	217.83	216.07	208.88	279.80	180.37	288.70	255.19	231.11
mpd.rand.mean	250.20	252.27	249.18	251.41	249.63	250.36	250.54	250.39	249.88
mpd.rand.sd	26.14	51.54	20.18	39.75	21.44	38.51	22.85	24.64	23.82
mpd.obs.rank	160	148	9	42	867	2	915	527	309
mpd.obs.z	-0.94	-0.67	-1.64	-1.07	1.41	-1.82	1.67	0.19	-0.79
mpd.obs.p	0.16	0.15	0.01	0.04	0.87	0.00	0.92	0.53	0.31
wt.mpd.obs	174.67	29.41	124.82	68.32	236.21	72.84	169.31	179.38	143.56
wt.mpd.rand.me	183.19	130.12	217.98	197.42	229.16	153.55	220.61	218.31	218.80
wt.mpd.rand.sd	51.54	59.46	43.65	61.82	41.76	48.01	44.35	50.85	46.28
wt.mpd.obs.rank	541	3	1	3	781	3	15	73	3
wt.mpd.obs.z	-0.17	-1.69	-2.13	-2.09	0.17	-1.68	-1.16	-0.77	-1.63
wt.mpd.obs.p	0.54	0.00	0.00	0.00	0.78	0.00	0.02	0.07	0.00
mntd.obs	98.49	102.45	64.18	55.99	85.57	50.47	80.71	93.41	77.12
mntd.rand.mean	104.33	148.64	93.03	125.88	95.08	125.02	97.55	101.64	100.93
mntd.rand.sd	18.75	41.93	13.45	30.19	14.56	29.54	15.20	16.57	16.47
mntd.obs.rank	389	132	14	6	277	2	147	325	72
mntd.obs.z	-0.31	-1.10	-2.15	-2.32	-0.65	-2.52	-1.11	-0.50	-1.45
mntd.obs.p	0.39	0.13	0.01	0.01	0.28	0.00	0.15	0.33	0.07
wt.mntd.obs	82.83	40.11	31.42	34.60	61.01	22.57	24.86	65.77	39.47
wt.mntd.rand.me	103.71	148.73	90.74	122.68	94.11	121.89	96.82	99.31	98.93
wt.mntd.rand.sd	55.40	85.80	35.69	54.47	28.26	67.93	35.24	37.50	34.61
wt.mntd.obs.ran	456	49	6	10	98	1	1	157	10
wt.mntd.obs.z	-0.38	-1.27	-1.66	-1.62	-1.17	-1.46	-2.04	-0.89	-1.72
wt.mntd.obs.p	0.46	0.05	0.01	0.01	0.10	0.00	0.00	0.16	0.01

Severson	*Round/Bullhead*	*Punished Woman Seeded*	*Punished Woman Grazed*	*Overland*	*McKillican*	*Lake Ketchum*	*Jacobson Fen*	*Hole in the Mountain*	*Gary Gulch W*
24	26	23	23	22	14	19	38	25	38
2158.05	1291.20	1199.60	1728.34	1406.45	923.86	1010.82	2900.33	1279.89	2280.35
1952.22	2073.33	1900.48	1888.74	1853.25	1308.18	1653.55	2724.37	2028.38	2741.50
352.68	364.73	357.16	349.36	356.77	297.45	333.11	387.86	355.82	382.19
658	3	3	425	81	46	4	625	3	150
0.58	-2.14	-1.96	-0.46	-1.25	-1.29	-1.93	0.45	-2.10	-1.21
0.66	0.00	0.00	0.43	0.08	0.05	0.00	0.63	0.00	0.15
1545.09	1114.31	1037.16	1292.02	949.94	681.67	696.81	1964.41	949.21	1566.44
1924.78	2028.17	1852.14	1835.43	1783.48	1281.84	1601.37	2655.36	1966.50	2687.48
-0.40	-1.45	-1.24	-0.83	-1.31	-1.08	-1.71	-0.67	-1.41	-1.13
0.39	0.02	0.04	0.17	0.02	0.06	0.00	0.24	0.02	0.06
946.01	630.15	656.70	657.63	635.09	555.63	530.43	1025.80	722.25	996.15
275.44	213.79	208.59	252.26	226.35	220.55	207.95	258.04	207.43	225.70
250.60	249.44	249.62	250.57	250.01	249.11	249.07	250.03	249.74	250.16
28.74	27.18	29.41	30.09	30.65	38.76	32.84	21.38	27.99	21.67
767	19	12	612	203	148	17	619	10	113
0.86	-1.31	-1.40	0.06	-0.77	-0.74	-1.25	0.37	-1.51	-1.13
0.77	0.02	0.01	0.61	0.20	0.15	0.02	0.62	0.01	0.11
106.05	160.89	158.92	174.42	140.56	68.38	99.86	175.22	116.46	136.32
167.32	213.68	209.82	206.31	203.53	168.79	207.47	205.34	202.96	208.40
54.39	48.41	49.86	57.57	57.73	60.98	57.85	51.49	50.05	43.89
27	28	34	157	25	7	3	128	5	3
-1.13	-1.09	-1.02	-0.55	-1.09	-1.65	-1.86	-0.59	-1.73	-1.64
0.03	0.03	0.03	0.16	0.03	0.01	0.00	0.13	0.01	0.00
115.98	62.91	65.25	87.94	86.91	82.06	67.48	105.03	71.57	90.55
110.26	107.53	111.31	110.86	113.01	128.81	117.96	95.13	108.69	95.55
20.61	18.73	21.62	21.81	21.70	29.40	25.39	14.07	21.21	13.98
616	8	12	151	112	49	13	754	29	359
0.28	-2.38	-2.13	-1.05	-1.20	-1.59	-1.99	0.70	-1.75	-0.36
0.62	0.01	0.01	0.15	0.11	0.05	0.01	0.75	0.03	0.36
46.86	39.68	38.08	36.78	37.97	48.30	36.95	56.87	45.75	43.47
108.47	106.30	111.59	111.17	111.20	125.79	115.71	94.12	106.63	94.72
62.30	43.20	46.80	46.50	47.48	64.06	45.76	43.71	46.57	43.33
102	9	8	12	8	57	4	166	37	54
-0.99	-1.54	-1.57	-1.60	-1.54	-1.21	-1.72	-0.85	-1.31	-1.18
0.10	0.01	0.01	0.01	0.01	0.06	0.00	0.17	0.04	0.05

	Sioux Prairie	Wilke
	34	48
	1715.18	3239.31
	2550.32	3245.12
	374.95	386.80
	2	426
	-2.23	-0.02
	0.00	0.43
	1272.96	2005.93
	2473.64	3137.35
	-1.54	-1.35
	0.01	0.05
	777.70	838.79
	217.19	246.15
	250.19	250.54
	23.85	18.43
	26	359
	-1.38	-0.24
	0.03	0.36
	180.29	184.56
	220.16	229.00
	47.44	44.79
	59	26
	-0.84	-0.99
	0.06	0.03
	68.62	93.71
	98.57	87.44
	15.96	11.53
	31	692
	-1.88	0.54
	0.03	0.69
	47.94	43.02
	97.29	87.54
	35.45	29.83
	27	21
	-1.39	-1.49
	0.03	0.02



Supplementary Figure 1: Uncorrected Faith's PD, MPD, and MNTD values across sites versus species richness for unweighted (top row) and abundance-weighted (bottom row) metrics. Spearman's correlation shown.



Supplementary Figure 2: Species richness of the tallgrass prairie of the Northern Great Plains by family over the growing season. Species occurrence data collected from the Consortium of Northern Great Plains Herbaria. Families ordered by species richness. (Polygon: 49.735908 -98.370117,42.274134 -98.326172,42.404066 -92.085938,44.163366 -92.920898,45.225458 -95.733398,46.721859 -96.392578,48.731625 -96.568359,49.536684 -97.183594,49.735908 -98.370117)

APPENDIX B

Supplementary Table 7: Voucher information for sampled specimens at Oak Lake FIeld Station and C.A. Taylor (SDState) Herbarium for DNA barcoding (Chapter 2).

Last	First	#	Date	*Species*	Locality	Herbarium	Collection
Anders	Paul	24	9/8/84	Eupatorium perfoliatum	T113N R51W S24 NW, Peg munky Run, Deuel Co.	OLFS	Anders 24
Anderson	Monte D.	2	6/12/83	Galium aparine	Oakwood Lake, Brookings Co.	OLFS	Anderson 2
Anderson	Monte D.	17	6/22/83	Medicago lupulina	T111N R50W S24 NE, SDSU Agronomy Farm, Brookings Co.	OLFS	Anderson 17
Anderson	Monte D.	9	6/12/83	Matricaria matricarioides	T112N R51W S5, Brookings Co.	OLFS	Anderson 9
Bauer	Daryl L.	42	8/18/86	Eupatorium rugosum	T12N R5E S34 SE, Minnehaha Co.	OLFS	Bauer 42
Beauzay	Patrick B.	296	7/17/95	Achillea millefolium	OLFS, Brookings Co.	OLFS	Beauzay 296
Beauzay	Patrick B.	323	8/8/95	Circuta bulbifera	OLFS, Brookings Co.	OLFS	Beauzay 323
Beauzay	Patrick B.	303	7/18/95	Plantago major	OLFS, Brookings Co.	OLFS	Beauzay 303
Beauzay	Patrick B.	282	7/17/95	Onosmodium molle	OLFS, Brookings Co.	OLFS	Beauzay 282
Beauzay	Patrick B.	352	8/8/95	Lysimachia ciliata	OLFS, Brookings Co.	OLFS	Beauzay 352
Beauzay	Patrick B.	330	8/8/95	Lobelia siphilitica	OLFS, Brookings Co.	OLFS	Beauzay 330
Beauzay	Patrick B.	257	7/13/95	Scirpus pallidus	OLFS, Brookings Co.	OLFS	Beauzay 257
Beauzay	Patrick B.	343	8/8/95	Setaria glauca	OLFS, Brookings Co.	OLFS	Beauzay 343
Beauzay	Gary	276	7/17/95	Trifolium pratense	OLFS, Brookings Co.	OLFS	Beauzay 276
Beauzay	Patrick B.	332	8/8/95	Cicuta maculata	OLFS, below Pioneer house	SDC	Beauzay 332
Beauzay	Patrick B.	322	8/8/95	Sium suave	OLFS, below Pioneer house	SDC	Beauzay 322

Beauzay	Patrick B.	327	8/8/95	Asclepias incarnata	OLFS, below Pioneer house	SDC	Beauzay 327
Beauzay	Patrick B.	326	8/8/95	Eupatorium maculatum	OLFS, below Pioneer house	SDC	Beauzay 326
Beauzay	Patrick B.	337	8/8/95	Solidago gigantea	OLFS, below Pioneer house	SDC	Beauzay 337
Beauzay	Patrick B.	330	8/8/95	Lobelia siphilitica	OLFS, below Pioneer house	SDC	Beauzay 330
Beauzay	Patrick B.	341	8/8/95	Cyperus odoratus	OLFS, T112N R47W S18 SW	SDC	Beauzay 341
Beauzay	Patrick B.	334	8/8/95	Oenothera biennis	OLFS, below Pioneer house	SDC	Beauzay 334
Beauzay	Patrick B.	351	8/8/95	Mimulus ringens	OLFS, below Pioneer house	SDC	Beauzay 351
Beauzay	Patrick B.	335	8/8/95	Impatiens capensis	OLFS, below Pioneer house	SDC	Beauzay 335
Beauzay	Patrick B.	317	8/8/95	Amorpha fruticosa	OLFS, N end of lake	SDC	Beauzay 317
Beauzay	Patrick B.	346	8/8/95	Lycopus americanus	OLFS, SW corner of lake	SDC	Beauzay 346
Beauzay	Patrick B.	342	8/8/95	Lycopus asper	OLFS, SW corner of lake	SDC	Beauzay 342
Beauzay	Patrick B.	347	8/8/95	Echinochloa muricata	OLFS, SW corner of lake	SDC	Beauzay 347
Beauzay	Patrick B.	325	8/8/95	Spartina pectinata	OLFS, below Pioneer house	SDC	Beauzay 325
Beauzay	Patrick B.	321	8/8/95	Polygonum amphibium	OLFS, below Pioneer house	SDC	Beauzay 321
Beauzay	Patrick B.	320	8/8/95	Polygonum lapathifolium	OLFS, below Pioneer house	SDC	Beauzay 320
Beauzay	Patrick B.	339	8/8/95	Polygonum punctatum	OLFS, below Pioneer house	SDC	Beauzay 339
Beauzay	Patrick B.	349	8/8/95	Agrimonia striata	OLFS, SW of lake	SDC	Beauzay 349
Beauzay	Patrick B.	319	8/8/95	Verbena hastata	OLFS, below Pioneer house	SDC	Beauzay 319
Bettross	Edward A.	51	9/6/86	Juncus dudleyi	2 mi S, 3 mi W of Marvin, Grant Co.	OLFS	Bettross 51
Bortnem	Robin	23	5/29/89	Mirabilis nyctaginea	T121N R50W S27, Marvin Gulch, Grant Co.	OLFS	Bortnem 23

Bortnem	Robin	2	4/26/89	Stellaria media	SDSU, Brookings Co.	OLFS	Bortnem 2
Bortnem	Robin	102	8/28/89	Solidago missouriensis	T110N R50W S35, Old Medary roadside, Brookings Co.	OLFS	Bortnem 102
Bortnem	Robin	110	9/18/89	Aster ericoides	T109N R50W S14, Brookings Co.	SDC	Bortnem 110
Bortnem	JM	sn	6/26/02	Dichanthelium oligosanthes	OLFS, T112N R48W S12 SW of SW	SDC	Bortnem sn
Christner	Tabitha	sn	6/18/09	Phleum pratense	43 18' 42.39' N 103 49' 5.04" W, Hwy 18 East of Edgemont, Brookings Co.	OLFS	Christner sn
Dirks	Brian J.	sn	9/7/88	Sporobolus compositus	Sexauer Park Brookings, Brookings Co.	OLFS	Dirks sn
Fairlee	Eric	54	6/3/97	Caragana arborescens	T127N R61W S14 S of NE, Hecla Sandhills, Brown Co.	OLFS	Fairlee 54
Fredrickson	Nicole L.	sn	9/9/97	Lemna turionifera	T109N R50W S36 SW, Brookings Co.	OLFS	Fredrickson sn
Genereux	Nick D.	sn	7/27/09	Elymus villosus	Sully Hill Game Preserve, T152 R65, Benson Co.	OLFS	Genereux sn
Hansen	Paul L.	852	9/3/81	Calamagrostis stricta	1.5 mi N Summit, Roberts Co	OLFS	Hansen 852
Jensen	Jill	sn	6/5/02	Zizia aurea	OLFS, NE of dining hall	SDC	Jensen sn
Jensen	Jill	sn	7/9/02	Apocynum cannibinum	OLFS, SW of shower station	SDC	Jensen sn
Jensen	Jill	sn	7/31/02	Asclepias verticillata	OLFS, W of shower station	SDC	Jensen sn
Jensen	Jill	sn	7/3/02	Echinacea angustifolia	OLFS, N of dining hall	SDC	Jensen sn
Jensen	Jill	sn	7/3/02	Echinacea angustifolia	OLFS, N of dining hall	SDC	Jensen sn
Jensen	Jill	sn	7/25/02	Helianthis maximilian	OLFS, W of shower unit	SDC	Jensen sn
Jensen	Jill	sn	7/25/02	Heliopsis helianthoides	OLFS, S of shower unit	SDC	Jensen sn

Jensen	Jill	sn	7/3/02	Ratiba columnifera	OLFS, N of dining hall	SDC	Jensen sn
Jensen	Jill	sn	5/30/02	Senecio plattnesis	OLFS, S of classroom	SDC	Jensen sn
Jensen	Jill	sn	7/31/02	Solidago canadensis	OLFS, E of Admin building	SDC	Jensen sn
Jensen	Jill	sn	6/24/02	Tragopogon dubius	OLFS, S of Admin building	SDC	Jensen sn
Jensen	Jill	sn	7/25/02	Veronia fasiculata	OLFS, S of shower unit	SDC	Jensen sn
Jensen	Jill	sn	6/24/02	Convolvus sepium	OLFS, N of flag pole	SDC	Jensen sn
Jensen	Jill	sn	8/13/02	Equisetum arvense	OLFS, N of Admin building	SDC	Jensen sn
Jensen	Jill	sn	5/30/02	Comandra pallida	OLFS, S of classroom	SDC	Jensen sn
Jensen	Jill	sn	5/30/02	Lithospermum angustifolium	OLFS, S of classroom	SDC	Jensen sn
Jensen	Jill	sn	7/9/02	Amorpha canescens	OLFS, S of shower unit	SDC	Jensen sn
Jensen	Jill	sn	6/24/02	Psoralea esculenta	OLFS, S of Admin building	SDC	Jensen sn
Jensen	Jill	sn	6/17/02	Trifolium pratense	OLFS, W of Admin building	SDC	Jensen sn
Jensen	Jill	sn	6/18/02	Vicia americana	OLFS, E of Admin building	SDC	Jensen sn
Jensen	Jill	sn	7/22/02	Petalotemon purpueus	OLFS, W of Admin building	SDC	Jensen sn
Jensen	Jill	sn	7/2/02	Lilium philadelphicum	OLFS, N of dining hall	SDC	Jensen sn
Jensen	Jill	sn	7/25/02	Bouteloua curtipendula	OLFS, S of shower unit	SDC	Jensen sn
Jensen	Jill	sn	6/18/02	Aquilegia canadensis	OLFS, E of Admin building	SDC	Jensen sn
Jensen	Jill	sn	7/9/02	Delphinium virescens	OLFS, S of shower unit	SDC	Jensen sn
Jensen	Jill	sn	6/18/02	Ranunculus macounii	OLFS, E of Admin building	SDC	Jensen sn
Jensen	Jill	sn	5/17/02	Geum triflorum	OLFS, E of dining hall	SDC	Jensen sn
Jensen	Jill	sn	6/24/02	Galium boreale	OLFS, N of flag pole	SDC	Jensen sn
Jensen	Jill	sn	6/3/02	Verbena stricia	OLFS, N of dining hall	SDC	Jensen sn
Johnson	James R.	419	6/26/97	Carduus nutans	OLFS, Brookings Co.	OLFS	Johnson 419
Johnson	James R.	420	6/26/97	Elaeagnus angustifolia	OLFS, Brookings Co.	OLFS	Johnson 420

Johnson	Emily	sn	6/21/10	Melilotus alba	OLFS, S of shower unit	SDC	Johnson sn
Johnson	Emily	sn	6/4/10	Melilotus officinalis	OLFS, S of shower unit	SDC	Johnson sn
Kanoute	Assetou	33	8/13/87	Elymus repens	T121N R50W S5 E, Grant Co.	OLFS	Kanoute 33
Kanoute	Assetou	92	8/11/87	Muhlenbergia cuspidata	T119N R51W S14 SW, Punished Woman Lake, Codington Co.	OLFS	Kanoute 92
King	Kevin	94	9/27/84	Apocynum cannabinum	T110N R50W S14 SE, Brookings Co.	OLFS	King 94
King	Kevin	103	9/19/84	Panicum virgatum	Oakwood Lakes, Brookings Co.	OLFS	King 103
Kjellsen	Mike	35	9/6/86	Glyceria striata	O' Farrell WPA, Near Marvin, Grant Co.	OLFS	Kjellsen 35
Kopp	Christopher W.	225	6/16/02	Koeleria macrantha	N 45 37' 38" W 102 30' 57", Grand River National Grassland, Perkins Co.	OLFS	Kopp 225
Larson	Gary	9922	9/23/83	Ambrosia psilostachya	Waterfowl Production Area	OLFS	Larson 9922
Larson	Gary	9917	9/23/83	Ambrosia artemisiifolia	T110N R51W S18 NE, Brookings Co.	OLFS	Larson 9917
Larson	Gary	sn	8/25/79	Helianthus grosseserratus	Aurora Prairie, Brookings Co.	OLFS	Larson sn
Larson	Gary	11732	8/20/96	Helianthus nuttallii	Hecla Sandhills, T128N R59W S18 W1/2 of NW, Marshall Co.	OLFS	Larson 11732
Larson	Gary	8980	6/30/83	Erigeron philadelphicus	T113N R50W S24 NE, Deuel Co.	OLFS	Larson 8980
Larson	Gary	11358	7/10/96	Calystegia macounii	Hecla Sandhills, T128N R59W S30 NE, Marshall Co.	OLFS	Larson 11358

Larson	Gary	11247	7/10/03	Calylophus serrulatus	Oakwood Lakes, T111N R52W S2 NW of NE, Brookings Co.	OLFS	Larson 11247
Larson	Gary	10982	8/10/92	Dichanthelium wilcoxianum	T1N R7E S5 NW, West Camp Rapid, Pennington Co.	OLFS	Larson 10982
Larson	Gary	11060	7/19/93	Dichanthelium acuminatum	T3S R5E S6 NE, Custer Co.	OLFS	Larson 11060
Larson	Gary	6680	8/15/81	Amphicarpa bracteata	W edge of Sica Hollow State Park, Marshall Co.	OLFS	Larson 6680
Larson	Gary	11240	7/1/03	Cornus sericea	T111N R51W S8 NW, Oakwood Lake Game Production Area, Brookings Co.	OLFS	Larson 11240
Larson	Gary	6963	9/11/81	Ceratophyllum demersum	0.5 mi N, 15.5 mi W Fort Pierre, Stanley Co.	OLFS	Larson 6963
Larson	Gary	11227	6/9/03	Celastrus scandens	Oakwood Lakes, Brookings Co.	OLFS	Larson 11227
Larson	Gary	9165	7/12/83	Carex utriculta	T123N R53W S16 SW, Waubay Nat'l Wildlife Refuge, Day Co.	OLFS	Larson 9165
Larson	Gary	sn	6/14/11	Carex tetanica	44 47' 26.2" N 96 37' 55.4" W, South Hamann Fen, Deuel Co.	OLFS	Larson sn
Larson	Gary	6396	5/31/80	Carex tenera	8 mi S of Brookings, Brookings Co.	OLFS	Larson 6396
Larson	Gary	11216	5/28/13	Carex sprengelii	T112N R51W S31 SW, Brookings Co.	OLFS	Larson 11216
Larson	Gary	sn	6/15/11	Carex sartwellii	44 46' 38.5" N 96 35' 43.0" W, Mud Lake, Deuel Co.	OLFS	Larson sn
Larson	Gary	9365	7/20/83	Carex molesta	T114N R47W S5 SE, Lake Cochrane, Deuel Co.	OLFS	Larson 9365

Larson	Gary	9270	7/15/83	Carex pellita	T126N R53W S33 SW, Cottonwood Lake, Marshall Co.	OLFS	Larson 9270
Larson	Gary	6395	5/31/80	Carex gravida	8 mi S of Brookings, Brookings Co.	OLFS	Larson 6395
Larson	Gary	11346	7/15/09	Carex granularis	OLFS, Brookings Co.	OLFS	Larson 11346
Larson	Gary	6393	5/31/80	Carex emoryi	8 mi S of Brookings, Brookings Co.	OLFS	Larson 6393
Larson	Gary	11132	6/14/96	Carex brevior	T128N R59W S5 W, Hecla Sandhills, Marshall Co.	OLFS	Larson 11132
Larson	Gary	11215	5/28/03	Carex blanda	T112N R51W S31 SW, Brookings Co.	OLFS	Larson 11215
Larson	Gary	9016	6/30/83	Lemna trisulca	T113N R53W S16 SE, Lake Norden, Hamlin Co.	OLFS	Larson 9016
Larson	Gary	9213	7/15/83	Galium trifidum	T124N R53W S26 NW, Pickeral Lake, Day Co.	OLFS	Larson 9213
Larson	Gary	6882	8/15/81	Galium triflorum	1 mi N, 8.5 mi E Lake City, west edge of Sica Hollow State Park, Marshall Co.	OLFS	Larson 6882
Larson	Gary	11335	7/1/09	Erysimum cheiranthoides	OLFS, Brookings Co.	OLFS	Larson 11335
Larson	Gary	11340	7/1/09	Eriophorum angustifolium	OLFS, Brookings Co.	OLFS	Larson 11340
Larson	Gary	11336	7/1/09	Geum canadense	OLFS, Brookings Co.	OLFS	Larson 11336
Larson	Gary	11639	8/13/96	Eleocharis erythropoda	T128N R59W S8 SE, Hecla Sandhills, Marshall Co.	OLFS	Larson 11639
Larson	Gary	6380	4/27/80	Dicentra cucullaria	2 mi N, 6 mi E of White, Brookings, Co.	OLFS	Larson 6380
Larson	Gary	9140	7/13/83	Platanthera hyperborea	T123N R53W S16 SW, Waubay Nat'l Wildlife Refuge, Day Co.	OLFS	Larson 9140

Larson	Gary	11328	7/1/09	Platanthera aquilonis	OLFS, Brookings Co.	OLFS	Larson 11328
Larson	Gary	11234	6/9/03	Penstemon albidus	T111N R52W S2 NE of NW, W Oakwood Game Production Area, Brookings Co.	OLFS	Larson 11234
Larson	Gary	11212	6/16/96	Oxalis violaceae	T128N R59W S18 NW NW, Hecla Sandhills, Marshall Co.	OLFS	Larson 11212
Larson	Gary	8724	6/8/83	Osmorhiza longistylis	T115N R47W S27 W, Cobb Creek, Deuel Co.	OLFS	Larson 8724
Larson	Gary	9041	6/30/83	Melilotus officinalis	T113N R53W S35 NW, Lake Albert, Hamlin Co.	OLFS	Larson 9041
Larson	Gary	11421	7/10/96	Medicago sativa	T128N R59W S8 NE, Hecla Sandhills, Marshall Co.	OLFS	Larson 11421
Larson	Gary	11426	7/11/96	Lycopus uniflorus	T128N R59W S7 SW, Hecla Sandhills, Marshall Co.	OLFS	Larson 11426
Larson	Gary	sn	7/27/92	Lobelia spicata	T128N R59W S1 NE, Marshall Co.	OLFS	Larson sn
Larson	Gary	11327	7/1/09	Liparis loeselii	OLFS, Brookings Co.	OLFS	Larson 11327
Larson	Gary	9967	5/30/84	Packera pseudaurea	T107N R50 W S36 NE, Sioux Prairie, Moody Co.	OLFS	Larson 9967
Larson	Gary	8920	6/24/83	Packera paupercula	T117N R50W S16 NE, Deuel Co.	OLFS	Larson 8920
Larson	Gary	sn	6/14/11	Scirpus microcarpus	44 47' 28" N 96 37' 42" W, Deuel Co.	OLFS	Larson sn
Larson	Gary	11264	7/1/96	Salix interior	T109N R49W S7 NW, Brookings Co.	OLFS	Larson 11264
Larson	Gary	7023	5/15/82	Salix amygdaloides	8 mi S of Brookings, Brookings Co.	OLFS	Larson 7023
Larson	Gary	10437	5/22/91	Rubus occidentalis	1 mi S and 3 mi W of Vermillion, Clay Co.	OLFS	Larson 10437

Larson	Gary	8885	6/24/83	Ribes americanum	T116N R49W S29 SE, S. Coteau Lake, Deuel Co.	OLFS	Larson 8885
Larson	Gary	6925	8/15/81	Rhus glabra	1 mi N, 8.5 mi E Lake City, west edge of Sica Hollow State Park, Marshall Co.	OLFS	Larson 6925
Larson	Gary	6912	8/15/81	Rudbeckia laciniata	6 mi N, 8.5 W Sisseton; Sica Hollow State Park, Roberts Co.	OLFS	Larson 6912
Larson	Gary	11227	6/16/96	Populus tremuloides	T128N R59W S19 W, Hecla Sandhills, Marshall Co.	OLFS	Larson 11227
Larson	Gary	11565	7/18/96	Polygonum aviculare	T128N R59W S6 SW, Hecla Sandhills, Marshall Co.	OLFS	Larson 11565
Larson	Gary	7019	5/12/82	Viola sororia	8 mi S of Brookings, Brookings Co.	OLFS	Larson 7019
Larson	Gary	11219	5/28/03	Viola pedafida	T112N R51W S31 NE, Goodfellow Waterfowl Production Area, Brookings Co.	OLFS	Larson 11219
Larson	Gary	9985	5/27/86	Viola nephrophylla	T115N R48W S6 S SW, Crystal Springs Ranch, Deuel Co.	OLFS	Larson 9985
Larson	Gary	11214	5/28/03	Viola canadensis	T112N R51W S31 SW, Brookings Co.	OLFS	Larson 11214
Larson	Gary	11538	7/17/96	Vernonia fasciculata	T128N R59W S31 SE, Hecla Sandhills, Marshall Co.	OLFS	Larson 11538
Larson	Gary	9999	5/29/86	Veronica peregrina	SDSU, Brookings Co.	OLFS	Larson 9999
Larson	Gary	10785	6/18/92	Veronica anagallis-aquatica	T3S R5E S6 NE, Custer State Park, Custer Co.	OLFS	Larson 10785
Larson	Gary	11559	7/18/96	Ulmus pumila	T128N R59W S6 SE, Hecla Sandhills, Marshall Co.	OLFS	Larson 11559

Larson	Gary	9117	7/9/83	Typha latifolia	T123N R53W S16 SW, Waubay Nat'l Wildlife Refuge, Day Co.	OLFS	Larson 9117
Larson	Gary	11386	7/10/96	Typha X glauca	T128N R59W S30 NW, Hecla Sandhills, Marshall Co.	OLFS	Larson 11386
Larson	Gary	11716	8/20/96	Toxicodendron rydbergii	T128N R60W S12 SE, Hecla Sandhills, Brown Co.	OLFS	Larson 11716
Larson	Gary	6651	8/19/80	Thalictrum dasycarpum	3 mi N and 7 mi W of Bruce, Brookings Co.	OLFS	Larson 6651
Larson	Gary	sn	6/15/11	Stellaria longifolia	44 46' 47.8" N 96 36' 05.1" W, Deuel Co.	OLFS	Larson sn
Larson	Gary	11508	7/16/96	Stachys palustris	T128N R59W S28 E, Hecla Sandhills, Marshall Co.	OLFS	Larson 11508
Larson	Gary	11341	7/1/09	Sphenopholis intermedia	OLFS, Brookings Co.	OLFS	Larson 11341
Larson	Gary	8750	6/8/83	Smilacina stellata	T115N R47W S9 NE, Gary Creek State Game Management Area, Deuel Co.	OLFS	Larson 8750
Larson	Gary	8733	6/8/83	Sisyrinchium campestre	T115N R47W S27 W, Cobb Creek, Deuel Co.	OLFS	Larson 8733
Larson	Gary	9926	9/23/83	Solidago rigida	Waterfowl Production Area, T111N R51W S28 SW	SDC	Larson 9926
Larson	Gary	11330	7/1/09	Carex cristatella	OLFS, 44 30' 54.7" N 96 32' 27.5" W	SDC	Larson 11330
Larson	Gary	11337	7/1/09	Carex hystericina	OLFS, 40 31' 23.5" N 96 32' 31" W	SDC	Larson 11337
Larson	Gary	11329	7/1/09	Carex stricta	OLFS, 44 30' 59.5" N 96 32' 26.2" W	SDC	Larson 11329
Larson	Gary	11345	7/15/09	Carex vulpinoidea	OLFS, 44 31' 23" N 96 32' 29" W	SDC	Larson 11345

Larson	Gary	11331	7/1/09	Populus x jackii	OLFS, 44 31' 23.5" N 96 32' 32.5" W	SDC	Larson 11331
Larson	Gary	11325	7/1/09	Mimulus glabratus	OLFS, 44 31' 23.2" N 96 32' 32.2" W	SDC	Larson 11325
Larson	Gary	11334	7/1/09	Juncus nodosus	OLFS, 44 31' 22" N 96 32' 27" W	SDC	Larson 11334
Larson	Gary	6850	6/19/81	Poa pratensis	1 mi. W of Aurora	SDC	Larson 6850
Larson	Gary	sn	9/12/97	Sorghastrum nutans	Oakwood State Park, restored prairie	SDC	Larson sn
Larson	Gary	11341	7/1/09	Sphenopholis intermedia	OLFS, 44 30' 54.7" N 96 32' 27.5" W	SDC	Larson 11341
Larson	Gary	11332	7/1/00	Geum aleppicum	OLFS, 44 31' 22" N 96 32' 27" W	SDC	Larson 11332
Law	Mark E.	105	8/2/84	Conyza canadensis	Brookings Co., Agronomy Farm, Brookings	OLFS	Law 105
Law	Mark E.	73	9/10/84	Helianthus maximiliani	Near Brookings	OLFS	Law 73
Law	Mark E.	93	6/20/85	Lepidium densiflorum	7 mi N, 1 mi E, 1 mi S Brookings, Brookings Co.	OLFS	Law 93
Law	Mark E.	103	8/2/85	Verbena bracteata	Agronomy Farm, Brookings Co.	OLFS	Law 103
Lehman	Chad P.	38	8/14/03	Pinus ponderosa	UTM Z13 616200 E/4828110 N, Custer Co.	OLFS	Lehman 38
Lehman	Chad P.	179	7/19/01	Stipa viridula	UTM Z13 619010 E/4823100 N, Custer Co.	OLFS	Lehman 179
Leoschke	Mark J.	1531	6/23/95	Cypripedium candidium	T122N R52W S 18 N SW NE SE, ca 1 mi NW of Ortley, Roberts Co.	OLFS	Leoschke 1531
McLead	Scott	sn	6/19/92	Rorippa palustris	T124N R55W S13 NW, Day Co.	OLFS	McLead sn
Millar	James B.	37	9/6/86	Epilobium leptophyllum	7 mi N of Waubay, Day Co.	OLFS	Millar 37
Millar	James B.	11	9/6/86	Salix alba	7 mi N of Waubay, Day Co.	OLFS	Millar 11

Mixon	Kevin	sn	7/22/91	Cirsium arvense	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	8/5/91	Allium stellatum	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	8/20/91	Liatris punctata	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	8/5/91	Grindelia squarrosa	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	7/5/91	Asclepias syriaca	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	6/12/91	Lathyrus venosus	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	6/22/91	Oxalis stricta	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	7/5/91	Scrophularia lanceolata	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	8/20/91	Ratibida columnifera	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	6/11/91	Zigadenus elegans	T109N R49W S10 SW, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	7/5/91	Urtica dioica	OLFS, Brookings Co.	OLFS	Mixon sn
Mixon	Kevin	sn	8/20/91	Silphium perfoliatum	OLFS, Brookings Co.	OLFS	Mixon sn
Monteith	Kyle	sn	8/5/08	Fragaria virginiana	45 16' 22.24" N 97 53' 46.17" W, Day Co.	OLFS	Monteith sn
Monteith	Kyle	sn	8/5/08	Taraxacum officinale	45 16' 22.24" N 97 53' 46.17" W, Day Co.	OLFS	Monteith sn
Ode	D.J.	sn	7/11/79	Agrostis stolonifera	Aurora Prairie, Brookings Co.	OLFS	Ode sn
Ode	David J.	27-Dec	6/6/12	Celtis occidentalis	Little Bend Natural Area, ca. 30 mi W of Onida, Sully Co.	OLFS	Ode 44192
Ode	D.J.	83-57	5/21/83	Carex meadii	T101N R49W S1 SW SW, Cactus Hills, Minnehaha Co.	OLFS	Ode 83-57
Ode	D.J.	82-11	6/4/82	Carex aquatilis	T115N R48W S6 S SE, Jacob Springs, Deuel Co.	OLFS	Ode 82-11

Ode	D.J.	sn	6/26/80	Fraxinus pennsylvanica	T121N R71W S18 SE, Ryman WPA, Edmunds Co.	OLFS	Ode sn
Ode	David J.	Feb-40	8/15/03	Euthamia graminifolia	T125N R51W S8 NW NW, Schmidt prairie, Roberts Co.	OLFS	Ode 14642
Ode	D.J.	84-102	7/12/84	Cryptotaenia canadensis	Gilley's Grove, 1 mi N and 1 mi E of White, Brookings Co.	OLFS	Ode 84-102
Ode	D.J.	84-97	7/12/84	Phryma leptostachya	Gilley's Grove, 5 mi E, 1 mi N and 1 mi E of White, Brookings Co.	OLFS	Ode 84-97
Ode	David J.	00-21	6/29/00	Pedicularis lanceolata	T122N R52W S19 S of NE, ca 0.6 mi W of Ortley, Grant Co.	OLFS	Ode 00-21
Ode	D.J.	84-107	7/13/84	Poa palustris	T113N R48W S7 S SW, Quail Prairie, Deuel Co.	OLFS	Ode 84-107
Ode	David J.	7-Feb	6/11/02	Ranunculus hispidus	T122N R52W S18 N SW NE SE, ca 1 mi NW of Ortley, Roberts Co.	OLFS	Ode 43868
Ode	D.J.	sn	8/1/79	Polygonum coccineum	T109N R49W S10 W of NW, Brookings Co.	OLFS	Ode sn
Orth	Mandy R.	sn	9/16/09	Hordeum jubatum	44 19' 7.95" N 96 46' 31.44" W, SDSU campus, Brookings Co.	OLFS	Orth sn
Pauly	Brian	sn	8/31/10	Sporobolus heterolepis	44 14' 09.41" N 96 59' 3.22" W, SW of Volga, Brookings Co.	OLFS	Pauly sn
Pengra	R.M.	P-16-75	6/16/75	Lathyrus palustris	T109N R49W, Trenton twp., Brookings Co.	OLFS	Pengra P-16-75
Peterson	Altermott	sn	6/26/02	Carex praegracilis	OLFS, S of classroom	SDC	Peterson sn

Peterson	Altermott	sn	6/26/02	Bromus inermis	OLFS, S of classroom	SDC	Peterson sn
Pooler	P.D.	173	6/15/85	Agoseris glauca	Oakwood Lakes, Brookings Co.	OLFS	Pooler 173
Pooler	P.D.	84027	6/30/84	Agropyron cristatum	Oakwood Lakes, Brookings Co.	OLFS	Pooler 84027
Pooler	P.D.	84996	7/19/84	Calystegia sepium	N of Brookings on Hi-way 77, Brookings Co.	OLFS	Pooler 84996
Pooler	P.D.	186	6/5/85	Astragalus crassicarpus	Oakwood Lakes, Brookings Co.	OLFS	Pooler 186
Pooler	Paul	84045	5/18/84	Ribes missouriense	1 mi e of Oakwood Lakes, Brookings Co.	OLFS	Pooler 84045
Pooler	Paul	84029	6/30/84	Polygonum biflorum	Oakwood Lakes, Brookings Co.	OLFS	Pooler 84029
Pooler	P.D.	84028	6/30/84	Verbena stricta	Oakwood Lakes, Brookings Co.	OLFS	Pooler 84028
Purinton	Jordan	sn	5/24/12	Glenditsia triacanthos	44 9' 6.7" N 99 55' 44.2" W, Lyman Co.	OLFS	Purinton sn
Reese	R. Neil	sn	5/23/00	Crataegus rotundifolia	OLFS	SDC	Reese sn
Riley	Steve	51	6/8/84	Ulmus americana	T110N R50W S24 NW, Brookings Co.	OLFS	Riley 51
Roberts	R. Evelyn	sn	7/10/71	Physalis virginiana	T123N R54W S32, Waubay Nat'l Wildlife Refuge, Day Co.	OLFS	Roberts sn
Roberts	R. Evelyn	73-8-19:1	8/19/73	Rumex orbiculatus	T122N R53W S15 SW SW, Day Co.	OLFS	Roberts 73-8-19:1
Roberts	R. Evelyn	72-5-28:6	5/28/72	Tradescanta bracteata	T123N R55W S13 S, Blocks Slough, Day Co.	OLFS	Roberts 72-5-28:6
Roemmich	Aurora	114	9/16/08	Artemisia frigida	Oakwood Lake, Brookings Co.	OLFS	Roemmich 114
Roemmich	Aurora	81	9/8/08	Andropogon gerardii	N 44 21.702' W 96 47.266', Brookins Co.	OLFS	Roemmich 81

Roemmich	Aurora	98	9/18/08	Gentiana puberulenta	44 43.067" N 96 30.028" W, Orchid Meadow WPA, Brookings Co.	OLFS	Roemmich 98
Roemmich	Aurora	259	9/3/09	Scutellaria lateriflora	44 11' 49.04" N 96 47' 17.17" W, Conservation Park, Brookings Co.	OLFS	Roemmich 259
Roemmich	Aurora	82	9/8/08	Setaria viridis	44 21.702' N 96 47.266' W, N Meadary Ave, Brookings Co.	OLFS	Roemmich 82
Sargent	Douglas	sn	9/1/88	Bidens frondosa	T109N R49W S7 NW, Brookings Co.	OLFS	Sargent sn
Sletten	Kris	303	7/1/83	Glyceria grandis	T109N R47W S33 NE, Brookings Co.	OLFS	Sletten 303
Sletten	Kris	305	7/1/83	Calamagrostis canadensis	T109N R47W S33 NE, Brookings Co.	OLFS	Sletten 305
Sletten	Kris	169	6/21/83	Eleocharis palustris	T113N R47W S9 NE, Fish Lake, Deuel Co.	OLFS	Sletten 169
Sletten	Kris	136	6/21/83	Phalaris arundinacea	T112N R47W S29 SW, Lake Hendricks, Brookings Co.	OLFS	Sletten 136
Sletten	Kris	273	6/25/83	Lysimachia thyrsiflora	T122N R51W S27 NW, Schuchard Waterfowl Production Area, Roberts Co.	OLFS	Sletten 273
Sletten	Kris	175	6/21/83	Vitis riparia	T113N R47W S9 NE, Fish Lake, Deuel Co.	OLFS	Sletten 175
Sletten	Kris	379	7/7/83	Sphenopholis obtusata	T117N R55W S11 NE, Long Lake, Codington Co.	OLFS	Sletten 379

Sletten	Kris	210	6/25/83	Sparganium eurycarpum	T120N R51W S26 SW, State Game Production Area, Grant Co.	OLFS	Sletten 210
Stahnke	April L.	sn	7/31/01	Quercus macropoda	OLFS, Brookings Co.	OLFS	Stahnke sn
Stahnke	April L.	sn	6/7/02	Osmorhiza longistylis	Sica Hollow, Roberts Co., SD	SDC	Stahnke sn
Stahnke	April L.	sn	7/13/00	Apocynum cannibinum	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/28/20	Asclepias incarnata	OLFS, N of highway	SDC	Stahnke sn
Stahnke	April L.	sn	8/15/00	Aster laevis	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	8/29/00	Helenium autumnale	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	8/16/00	Kuhnia eupatorioides	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/17/00	Liatris aspera	OLFS, SE of shower area	SDC	Stahnke sn
Stahnke	April L.	sn	10/7/99	Ratiba columnifera	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	8/15/00	Solidago mollis	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	8/16/00	Symphoricarpos occidentalis	OLFS, towards end of peninsula	SDC	Stahnke sn
Stahnke	April L.	sn	5/28/00	Lonicera tatarica	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/15/99	Typha angustifolia	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	5/4/00	Lithospermum canescens	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	5/15/00	Lithospermum incisum	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/13/00	Psoralea argophylla	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/13/00	Dalea candida	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/10/00	Dalea purpurea	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/15/99	Monarda fistulosa	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	7/10/00	Stachys alustris	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	6/25/02	Anemone cylindrica	Sioux Prairie, Moody Co.	SDC	Stahnke sn
Stahnke	April L.	sn	5/19/00	Anemone patens	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	8/10/99	Rhamnus cathartica	OLFS, peninsula	SDC	Stahnke sn
Stahnke	April L.	sn	5/4/00	Amelanchier alnifolia	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	8/3/99	Prunus americana	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	5/15/00	Prunus virginiana	OLFS	SDC	Stahnke sn
Stahnke	April L.	sn	6/17/99	Rosa arkansana	OLFS	SDC	Stahnke sn

Steinauer	Gerry	sn	6/2/87	Cardamine bulbosa	T115N R48W S3 SE, Deuel Co.	OLFS	Steinauer sn
Taylor	C. A.	7506	8/29/51	Artemisia ludoviciana	Lake Poinsett, Brookings Co.	OLFS	Taylor 7506
Taylor	C. A.	sn	5/23/63	Aesculus glabra	Cultivated, Brookings, Brookings Co.	OLFS	Taylor sn
Taylor	C. A.	sn	5/14/70	Juniperus virginiana	escaped from cultivation, Oakwood Park, Brookings Co.	OLFS	Taylor sn
Taylor	C. A.	sn	6/25/63	Erigeron strigosus	T109N R49W S10 SW of NW, Brookings Co.	OLFS	Taylor sn
Taylor	C. A.	11799	10/9/80	Capsella bursa-pastoris	Brookings, Brookings Co.	OLFS	Taylor 11799
Taylor	C. A.	7540	6/8/52	Hydrophyllum virginianum	Sioux State Park, Dell Rapids, Minnehaha Co.	OLFS	Taylor 7540
Taylor	C. A.	sn	2/5/94	Picea glauca	Cultivated, Brookings, Brookings Co.	OLFS	Taylor sn
Taylor	C. A.	11723	9/15/77	Lycium barbarum	SDSU, Brookings Co.	OLFS	Taylor 11723
Troelstrup	Nels	sn	6/15/02	Zizia aptera	OLFS, west of dining hall	SDC	Troelstrup sn
Troelstrup	Nels	sn	7/25/02	Asclepias verticillata	OLFS, W of shower station	SDC	Troelstrup sn
Troelstrup	Nels	sn	8/19/04	Lygodesmia juncea	OLFS, T112N R48W S13 NW of NE	SDC	Troelstrup sn
Troelstrup	Nels	sn	8/26/04	Gentiana andrewsii	OLFS, T112N R48W S13 NW of NE	SDC	Troelstrup sn
Troelstrup	Nels	sn	5/30/02	Lithospermum canescens	OLFS, NW of dining hall	SDC	Troelstrup sn
Troelstrup	Nels	sn	5/30/02	Lathyrus polymorphus	OLFS, NW of dining hall	SDC	Troelstrup sn
Troelstrup	Nels	sn	9/4/04	Mentha arvensis	OLFS, T112N R48W S12 SW of SW	SDC	Troelstrup sn
Troelstrup	Nels	sn	6/25/02	Phlox pilosa	OLFS, T112N R48W S12 SW of SW	SDC	Troelstrup sn
Troelstrup	Nels	sn	6/15/02	Anemone canadensis	OLFS, west of dining hall	SDC	Troelstrup sn

Troelstrup	Nels	sn	5/15/02	Caltha palustris	OLFS, T112N R48W S12 SW of SW	SDC	Troelstrup sn
Unkenholz	Eric G.	sn	9/12/97	Rumex crispus	T111N R50W S34 NW, Mehegan State GPA, Brookings Co.	OLFS	Unkenholz sn
Van Sickle	Steve	586	9/15/82	Elymus virginicus	T109N R50W S36 SW, Brookings Co.	OLFS	Van Sickle 586
Van Sickle	Stephen	536	8/30/82	Schizachyium scaparium	Oakwood Lakes, Brookings Co.	OLFS	Van Sickle 536