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Vegetation Surveys in Southern Minnesota Prairies: Management, Invasive Species and Future Directions

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Vegetation Surveys in Southern Minnesota Prairies: Management, Invasive Species and Future Directions

By

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ABSTRACT

This paper is formatted into two chapters: a general introduction on prairies, management, and this study (Chapter 1), and the study formatted for submission to a journal for publication (Chapter 2). To manage habitat loss in Southern Minnesota prairies, and subsequent ecological damage, private and public individuals have responded with restoration. This study investigated the use of an accepted vegetation monitoring tool to survey prairies (N=31) in Southern Minnesota during June/July (2019), targeting peak growing season to see whether restored prairies had lower invasive species richness, and relatively greater native richness. We hypothesized that restored prairies would have higher species richness, fewer invasive species and lower phylogenetic diversity. A subset (N=11) were then re-surveyed in August (2019). We found that composite invasive species abundance score (CISA) did not vary significantly between restored and remnant prairies, but percent natural vegetation (%PNV) was significantly higher on restored prairie sites. Interestingly, we found a significant increase in species richness between June/July and August – further supported by a significant difference in %PNV for the two sampling periods, where more native species, and a higher %PNV score, were found in August. We found that management strategies (categorized in three groups: fire, mechanical, and chemical) did not vary significantly between restorations and remnants: neither management type, nor frequency, were significantly different. However, we did find some species-specific effects, as *Melilotus officinalis* coverage percentages increased significantly with increasing site area; *Berteroa incana* coverage percentages decreased significantly with increased species richness; and *Melilotus alba* coverage percentages increased significantly the longer a site had gone without mechanical management, and chemical management. We found that restored prairies scored significantly higher in three Nature Conservancy metrics: Landscape Diversity, Resilience, and Local Connectedness. Moreover, our phylogeny, consisting of 374 species, led to significant results as well. Significantly, we found increasing prescribed burn frequency led to increases in phylogenetic diversity. Moreover, we found that higher June/July species richness was positively correlated with higher phylogenetic diversity, but not CISA values, indicating that this diversity was not due to invasive or non-native species.

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CHAPTER 1: INTRODUCTION

The Minnesotan Prairie Ecosystem

This paper is formatted into two chapters: Chapter 1-General Introduction, and Chapter 2-Study formatted with the intent to submit for publication.

Prairies are an ecosystem that historically covered much of the American Midwest. Within this region lies Minnesota, a state that has four biomes: coniferous forest, tallgrass aspen parkland, deciduous forest, and prairie grassland-all of which draw the eye and countless visitors year-round. However, prairies serve an even more important purpose ecologically. Prairie systems are incredibly diverse (Judd et al., 2007) and robust, and provide such ecosystem services as are necessitated by the multitude of organisms that have historically relied upon them. Prairie vegetation is one of the most important, and easily recognizable, features of this landscape.

Characterized by an assemblage of forbs and grasses, with minimal woody vegetation, prairies are complex and striking systems. Historically, the tallgrass prairie system covered approximately 69 million hectares of land in the central United States region (Corpstein et al., 2014). However, due to a variety of variables, including human expansion and interference, habitat degradation, and fragmentation, the current space occupied by this once significant biome is estimated at less than 1% this value (Jangid et al., 2010, Rowe et al., 2013). In part because of this, a dedication and love for the natural world has inspired a great number of people to campaign for the remaining remnant prairies to be safeguarded from further degradation, and for suitable land to be converted to prairie, in an effort to maintain some of the ecological functions and natural wonder they provide.

The prairie that has survived until the present day can arguably be given one of two identifiers: remnant or restored. Remnant prairies are more rare than restored prairies, because restored prairies have not been as protected from human interference. Restorations are prairies that generally fit one of two descriptions. One: prairies that, for one reason or another, were cultivated or otherwise altered through human interference until they were no longer prairies. Often, the result, particularly in the Midwest United States, was cropland. However, after a length of time, individuals have decided to either return previously cultivated or otherwise degraded prairie to a natural state. The second path are restored prairies that were actively managed for on a plot of land deemed suited for this purpose; they had never been prairie before, but were managed and grown until they became prairie. Restorations tend to have lower species richness, lower native plant species richness, and higher exotic species richness (Hillhouse et al., 2011) as well as lower phylogenetic diversity (Barak et al., 2017). Furthermore, restoration success depends on feasible and effective management for promoting positive native populations, and for reducing impacts of non-native and invasive species (Trowbridge et al., 2016).

When one considers the great number of ecological hazards that prairies have historically faced, and the increasing public interest in their protection and creation, it follows then that there would be similar concern for areas of improvement and potential threats to these ecosystems. In the modern world, there are a significant number of considerations regarding prairie managements, including manpower, public interest, the associated costs of upkeep and creation. Moreover, there is the resounding question, *what works?* What seed mixes are best and what outcomes should inform species choice within those mixes (Bach et al., 2011)? Should they be tailored depending on the outcomes desired from the site, and if so, how should this be done? Once the prairie is in its early stages of management, what management is most efficacious, on what intervals, and with what goals in mind? There are an enormous number of questions associated with properly following through with these projects, and as more research is published on restoration practices, there are arguments that we should focus on returning to as natural a state as we can achieve, with some focused improvements (Bach et al., 2011). The answers to all of these questions, and considerations, hold practitioners' interest regardless of background. After all, the hope shared by all who love prairies, is that they be restored to a significant extent of their former glory.

Threats to Plant Diversity and Richness

One of the major concerns when one is discussing an ecosystem-at-risk is the degree to which human alteration is allowable, and what potential negative outcomes may arise from such interference. In most instances, this interference is management; for instance, bison (*Bos bison*) no longer walk the plains as they did historically, and fire is heavily controlled, lest it become an issue for the human development invariably nearby. Both bison (*Bos bison*) and fire are critical prairie management techniques (Knapp et al., 1999) that increase heterogeneity and diversity on prairies. However, because these two management techniques are either lacking or severely controlled, this poses potential threats to the upkeep of existing prairies and the management plans for new prairies. With this kind of interference, and progressively more destructive practices, humans have established prairies among the ranks of the most endangered systems in the United States (Krock et al., 2016), and have successfully limited associated seed stocks, causing a cascade of other issues.

Additionally, invasive species cause damage to the ecosystems they invade (Clinton, 1999). Once seed has been established in the seedbank, we see evidence that non-native seed species can out-pace and over-whelm native species, which is a limiting factor in restoration success (Zylka et al., 2016). Interestingly, we do see research (Larson et al., 2011) indicating that invasive species that fulfill similar niche requirements to native species are less likely to establish than their invasive counterparts with differing functional traits. We also see that trait overlap can become problematic, as unnatural competition is occurring and may be detrimental to the success of desired, native species in these ecosystems (Stanley et al., 2008). Evaluations ought to account for the varying outcomes of differing restorations, as well as acknowledge the importance of land use, management, and restoration practices (Millikin et al., 2015).

One of the major outcomes of management regimes had been the control of non-native and invasive plant species; one of the most concerning issues that we face in the protection of the prairie ecosystem, and particularly restorations (Stanley et al., 2008). Burning regimes are necessary (Brye et al., 2002) as the addition of fire may be able to remove species that did not adapt to fire in the same way that prairie species did. Many of these non-native species are considered undesirable, though there could be arguments to include some based on their potential for bringing new roles to the ecological table (availability for birds and small mammals [such as nesting material and cover from potential predation], desirability for pollinators, etc.). However, there need to be frank discussions balancing the potential positive aspects with any hazardous aspects (e.g. outcompeting native species, potential for forming monocultures, acting as a vector for disease).

Management: Methodologies and Outcomes

While management is intricately tied to all prairies, it is particularly significant in restorations, as a necessary consideration from the inception of the restoration project. While restorations tend to move quite quickly in the beginning, it is worth highlighting that there are significant changes (Brye et al., 2002) that occur within the natural succession until the variables balance in the first few seasons after the initial restoration has begun. There is also a desire for increased public awareness, transparency, and ease of information regarding prairie restorations (Lieberman et al., 2018), throughout the process and extending into the future.

Interestingly, we have seen evidence that restoration projects exhibit better species richness than their remnant counterparts (Trowbridge et al., 2017); this could be due to some aspect(s) of the management regimes and routine methodologies adopted by site managers. Often, we see managers use a variety of different management techniques to promote desired community characteristics (such as native species), and to reduce or remove undesirable community characteristics (such as invasive species). These techniques could include mechanical options, like cutting, hand-pulling, grazing, mowing, and so on. However, there are also chemical options (such as the application of pesticides). Finally, there is also the use of fire (prescribed burning). Moreover, we do see that different plant species can benefit from a certain degree of outside disturbance (Corpstein et al., 2014) as well as exhibit fidelity to particular conditions.

We see that, through human intervention via management, prairies can exhibit increased diversity, productivity, soil moisture and decreased levels of non-native species (Foster et al., 2015). However, this does not occur in a vacuum: every prairie, regardless of its' site history and management, is also surrounded with other land, be it human habitat, or perhaps more likely, agricultural land. Regardless, there need to be accommodations made for the state of surrounding lands and potential threats coming from those areas when managers plan for prairies (Rowe et al., 2013); they tend to have significant effects on management outcomes. After all, location and urban proximity, as well as nearby land-use practices can become problematic (Kricsfalusy et al., 2015). This can be difficult as prairies are complex ecosystems; how can managers correlate specific variables to specific outcomes, for good or ill? It can be very difficult, particularly over significant periods of time, to discern which variables correlate with specific results (Brye et al., 2002). We do know that there can be significant correlations that do occur between management and outcomes, even back into time, and that historical variables continue to play positive parts in the outcomes of restorations (Galatowitsch et al., 1998).

Probably the most charismatic, well known and widely used prairie management technique is the use of fire. Prairies are fire adapted ecosystems, meaning that developing burning regimes is an important restoration and maintenance method (Brye et al., 2002). Moreover, they are effective in turning over nutrients, as well as destroying invasive plant species (Heslinga et al., 2010). We see this particularly for woody vegetation, which is known to slowly degrade prairie ecosystems over time. However, fire regimes may also be one of the most difficult management techniques to adopt: it is expensive, risky, and intensive work. Nearby landowners may object to the use of fire, perceiving it as a threat; if managers lose control of a blaze, no one argues that the results could be tragic. However, in many cases now there are strict regulations, often requiring extensive fire safety planning and a well-trained suppression crew; burn regimes in the modern day are carefully planned, intensely controlled occurrences to minimize risk and maximize reward.

Phylogenetic Distance.

In recent years, phylogenetic distance has gained import as an indicator of ecosystem health beyond such measures as species richness, diversity, and so on because it can function as a measure of biodiversity in a system (Kembel et al., 2006; Flynn et al., 2011). In this study, we utilize Faith's phylogenetic diversity (1992), where the distances are determined using cladistic and taxonomic information. This is an important measure, because we can see diversity in phylogenetic health that may not be reflected in other measures – for instance, it is possible that species richness would not differ significantly between sites, but the phylogenetic distance would (Barak et al., 2017). In this scenario, it is worth noting that though the number of species are similar, the diversity represented by their evolutionary history may hold the key to identifying further environmental factors, or tie into differences in quality between sites. Moreover, phylogenetic diversity can serve as a proxy for novel features displayed by members of the tree (Faith, 1992), and evolutionary diversity.

Both species richness and phylogenetic diversity are measures of how diverse any given system is, and are intricately tied. Calculating Faith's (1992) phylogenetic diversity is both a measure of how many species are present (species richness), and how evolutionarily distinct they are from each other (phylogenetic diversity)-larger values are desirable, because they imply both an increase in species richness, and evolutionary distance. Both of these measures can therefore be expected to correlate with each other. Moreover, we see that species diversity may be related to prairie stability over time (Polley et al., 2007), and increases in this measure may aid in the ability of plant communities to persist with minimal property shifts. It is the potential for species richness and phylogenetic distance to describe diversity that led to their adoption in this study.

In conclusion, with prairie restoration growing in popularity in the last 50 years, it is increasingly important to understand what management techniques aid in these projects, and what possible plant invaders are a threat to the habitat's subsequent vegetative biodiversity. This project was designed to add to the existing body of knowledge regarding prairie restoration and potential for avoiding non-native and invasive plant species invasion in these ecosystems. To this effect, it was hypothesized that restored prairies would have lower invasive species richness, and would score better on the Bohnen & Galatowitsch (2016) metric (lower invasive species presence, and relatively more natural vegetation) due to increased human activity (management and restoration activities) acting as an unnatural selector. However, it was also hypothesized that remnant prairies would have greater phylogenetic diversity, in line with the findings of Barak et al. (2017), and that increased area would correlate with lower invasive species richness. Increased management frequency is also thought to correlate negatively with invasive plant species presence on these prairies (Stanley et al., 2008).

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CHAPTER 2: VEGETATION SURVEYS IN SOUTHERN MINNESOTA PRAIRIES: MANAGEMENT, INVASIVE SPECIES AND FUTURE DIRECTIONS

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INTRODUCTION

In the United States, one of the ecosystems that has been hardest hit by the advent of modern life (human expansion and land degradation) is the prairie. Historically widespread in the Midwest region, this incredibly diverse ecosystem has been whittled away for years. It is estimated that the central United States region had ~ 69 million acres of prairie (Corpstein et al., 2014) and now, less than one percent of this prairie remains (Jangid et al., 2010; Rowe et al., 2013). In Minnesota, this loss is particularly clear – in 2017, there is just ~250,000 acres of prairie remaining (Chaplin, 2018). Naturally, any time this kind of habitat loss is documented, there are real concerns about ecosystem goods and services, and how well species are able to adapt to disturbances, if they can at all. In more recent history, there are increasing numbers of people responding to this issue – both private citizens, as well as governmental agencies are intervening in various forms, including preserving remnant, unaltered territory, as well as restoration activity. This has been seen in various forms, such as the United States Department of Agriculture's (USDA) Conservation Reserve Program (CRP) and Conservation Reserve Enhancement Program (CREP); we also see Minnesota State's Legacy Funds and Reinvest in Minnesota (RIM) as examples of more localized, regional efforts. All of these programs are designed, and intended, to protect and invest in natural areas; specifically, endangered areas such as native grasslands and wetlands, among some others.

While remnants are designated as areas unaltered, or at the least as minimally altered by humans over time as possible, there are also restored habitats to consider. Restoration is a process intended to restore altered, degraded, destroyed or otherwise problematic habitats as close as possible to their prior, natural condition (Millikin et al., 2015). This is a complicated, intricate, and delicate process regardless of the scale and scope of planned interference. In prairie work, variables like planting method (Larson et al., 2011), seed mix richness (Larson et al., 2011), invasive species management (Larson et al., 2011) among other types of management, seed source and composition (Klopf et al., 2013; Grman et al., 2020) are among many important considerations. Moreover, these considerations require further planning based on man-power availability, funding, and accessibility.

Typically, the goal of restoration is to create as high quality a site as is possible, and to maintain that condition. This can be a complex under-taking, particularly over long periods of time. This is particularly true for prairie restorations because seed bank dynamics have the potential to persist for one or more seasons, depending on the species (Walck et al., 2005). Moreover, the progress of the plant community helps to dictates the speed of the restoration – which, by necessity, lasts for years before establishment, and continues afterwards in the form of management regimes. This process can be disrupted, or even degraded, by non-native and/or invasive plants – they are a significant threat to the prairie ecosystem (Stanley et al., 2008).

Degradation of biodiversity is a major concern in these systems. With the introduction of non-native and invasive species, we see a pattern of lowered vegetation diversity and structure, as well as fire regime, soil character and others (Stanley et al., 2008). It has also been established that restorations have lower biodiversity than remnants, as well as lower phylogenetic diversity despite having comparable species richness (Barak et al., 2017). This may be because many prairies are works-in-progress, and it may take significant periods for planted species to establish. It then follows that the biodiversity of prairies (both restored and remnant) in both phylogenetic diversity and species richness metrics, management histories, non-native and invasive species presence are all important considerations when determining whether a prairie is facing ecological threats.

There are a variety of ways to determine whether, and with what severity, a site is under threat – including surveying. Vegetative surveys are critical tools in restoration work, and are well supported and documented in literature (Corpstein et al., 2014; Bohnen et al., 2016; Lieberman et al., 2018). One such example is the Legislative-Citizen Commission on Minnesota Resources' (LCCMR) 2018 accepted survey methodology (Bohnen & Galatowitsch, 2016). This methodology relies on a series of timed meanders, to create master lists of all species found on surveyed lands, as well as their respective coverage percentages. We were interested in whether restored prairies had comparable vegetation to remnant prairies; to see whether restorations are able to meet remnant site quality, and to analyze how they achieved this state.

To this end, our goals were to survey vegetation present (and compile a master list of all found species per site, including cover percentages, and their designation as native, non-native or invasive), and to document whether there are potential degradation issues with non-native and/or invasive species. Finally, we wanted to see whether species richness, site history, management regimes, and phylogenetic diversity correlated with these surveyed vegetation community metrics. One of the major goals for this study was to document potential variables that indicate high-quality restorations for managers, as well as connect that desired state to actionable steps such as management types, frequencies and potential on-site variables that managers may encounter in Southern Minnesota.

Due to the large difference in human activity and site history differences between remnant and restored sites, we hypothesize our surveys to reveal four things. First, that restored prairies would have significantly more management occurring on site, which would lead to reductions in undesirable non-native and invasive species. Second, that restored prairies in Southern Minnesota would have lower invasive species richness, and would score better on the Bohnen & Galatowitsch (2016) metrics (lower invasive species presence, and relatively more native vegetation) compared to remnant prairies, because of increased management. Third, that restored prairies would have significantly more management occurring on site, which would lead to reductions in undesirable non-native and invasive species. Fourth, we hypothesize that remnant prairies would have greater phylogenetic diversity, in line with the findings of Barak et al. (2017) due to plant community preservation efforts, and that increased area would correlate with lower invasive species richness. Moreover, increased management frequency is also thought to correlate negatively with invasive plant species presence on these prairies (Stanley et al., 2008), which we hypothesized would occur in Southern Minnesota prairies.

METHODS

Site Descriptions

To determine the prairie quality and correlative management practices, we surveyed a selection of 22 prairie locations in southern-to-mid-Minnesota (Figure 1), which were further separated based on management and site history, leading to 31 individual prairies (Table 1). These prairies were surveyed from June through July 2019. Four locations, consisting of 10 prairies, are privately owned and belong to members of the Many Rivers chapter of The Prairie Enthusiasts. The remainder are under the authority of the Minnesota Department of Natural Resources and the Nature Conservancy. Most sites are in close proximity to agricultural land, and all but the private prairies are publicly accessible. In this study, we also analyzed data collected later in the growing season; those surveys were conducted on a subset (N=11, Table 2) of the original 31 prairies during a later time frame (original: June/July, follow-up: August) which allowed for an analysis based on a comparison between different periods in the growing season.

It is also worth highlighting that the 2019 growing season was delayed to an unusually wet, cool start to the season – compared to the historic average of growing degree days (gdd) for our region of study (~800 gdd by the end of June, 10º C base) we saw ~700 gdd (as recorded by University of Minnesota, Lamberton).

Survey Protocol

To get a representative sample of the vegetation present in the selected prairies, the survey protocol outlined by Bohnen and Galatowitsch (2016) was utilized. This survey method utilizes timed meander sampling; it is a highly adaptable methodology for developing an assessment of vegetation found in an area. Depending on the presence or absence of vegetation zones and acreage, a set number of timed meanders were determined for the area(s) of interest. After determining the number of meanders per site, each set of routes were divided to cover as representative a series of locations through the site as a whole. The base time for one meander is set at 30 minutes; however, this time was paused whenever the surveyors had to move between areas, or when an unknown species needed to be identified. During the sampling time, surveyors moved separately throughout the prairie, covering ground while identifying and taking note of all plant species encountered during the duration. As such, a list of species encountered and basic taxonomic information was compiled, detailing the meander when it was observed, as well as an approximation for the percentage of prairie the plant was present on.

This process was repeated for each of the 22 prairie locations (Figure 1), during June and July in 2019, in order to survey during peak growing season when most vegetation has enough growth (and potentially fruit or flower) to aid in species identification (Bohnen & Galatowitsch, 2016). Several of the locations had multiple prairies on site which were surveyed separately. During this process and the subsequent fall, managers were contacted to determine and compile site history and management history (2009-2019) for each prairie. Each site was categorized as follows: restored prairies had been non-prairie land for some length of time, and were in the process of being restored to prairie; remnant prairies had been prairie historically, and were unaltered from that state.

Phylogeny

In contrast to taxonomic species richness, we also calculated phylogenetic diversity (also called phylogenetic distance, or PD; Faith, 1992) to provide a metric of community evolutionary diversity, which has been shown to aid management decisions (Barak et al., 2017). This analysis relies upon the Smith & Brown (2018) tree ("ALLOTB.tre") which contains 353,185 taxa. Due to its large size, we pruned this tree (Appendix; Code 1) to only include taxa that were found during surveying, and due to some missing species (present in our surveys, but not represented at a species-level in the ALLOTB.tre file), we needed to create polytomies at the genus level for species not already present in the code via grafting. This allowed us to graft missing taxa onto existing genera. This ultimately led to the 374 seed plants (Figure 2) in the master phylogeny (Appendix; Code 2) that we used for analysis – some species were excluded (such as species within *Equisetum*) as they are not included as seed plants, but these were minimal in representation in the flora. Once pruning and grafting were completed, we calculated Faith's (1992) phylogenetic diversity (or phylogenetic distance) values for each site (Appendix; Code 3). This was completed utilizing R version ≥3.6.2 (R Core Development Team, 2020) with packages: APE v.5.4-1 (Paradis & Schliep, 2020), ADEPHYLO v.1.1-11 (Jombart et al., 2017), GDATA v.2.18.0 (Warnes et al., 2017), GEIGER v.2.0.6 (Harmon et al., 2015), NLME v.3.1-149 (Pinheiro et al., 2020), PEZ v.1.2-2 (Pearse et al., 2020), PHYLOBASE v.0.8.10 (Hackathon et al., 2020), PHYTOOLS v.0.7-70 (Revell, 2012), and PICANTE v.1.8.2 (Kembel et al., 2020).

Statistical Analyses

For all analyses, the level of significance was set at $p \leq 0.05$, and JMP Pro version 14 was utilized.

CISA and %PNV Scores. Scores were given to invasive species percent coverage (CISA) and percent natural vegetation (%PNV) according to the metric detailed by Bohnen and Galatowitsch (2016). These scores give an approximate indicator of prairie vegetative health, in order to rank sites based on the presence or absence of quality indicators (natural species and non-native to invasive species). CISA data failed normality, and so was log-transformed to meet that criteria before being analyzed via one-way analysis of variance (ANOVA).

Site History. To determine whether there were any potential significant relationships between site history, and other pertinent variables (e.g. management history, species richness, presence of invasive/non-native species, and TNC scores) a principle component analysis (PCA) was run using JMP. Variables indicating the potential for significant relationships were then tested for significance using one-way ANOVA, and Pearson's chi-squared and are included under respective headings.

Site Quality. According to the Bohnen & Galatowitsch (2016) metric, the CISA and %PNV scores and resulting midlines create four quadrants, denoting the quality of the surveyed sites with three distinct designations: high, medium, and low quality.

Management History. Management history was collected through discussion with site managers; talks began during the 2019 summer field season and lasted through the end of fall 2019. Information requested consisted of the frequency of management within the last decade (2009-2019); three management types were detailed: mechanical, chemical and fire. Mechanical management was defined as any manpower-based activity (cutting, hand-pulling, grazing, etc.); chemical management consisted of any application of herbicides on-site (including large-scale and hand-applications). Fire, as a distinctly significant management tool in the upkeep of prairie was considered a separate category. All frequencies were analyzed as the number of times a specific management category occurred on the site during the time frame (2009-2019). The subsequent analysis was a multiple regression to test whether any correlations existed between the three management history variables and CISA, as well as %PNV score.

There was the potential for ownership of the prairies surveyed to potentially have an effect on some of these quality metrics; for analysis, they were specified as public (belonging to the Minnesota Department of Natural Resources, and The Nature Conservancy) or private (owned by private Minnesota residents). In the original surveys, 10 of the 31 prairies were owned by private individuals, and the rest were public lands. For the August follow-up surveys, of the 11 total, five were public lands and six belonged to private individuals. To determine whether there was a difference (with regards to CISA, %PNV, and species richness) between public and privately managed prairies, one-way ANOVA tests were used.

To test whether there were any significant differences between management history (both frequency of management types, as well as the years since the last application) and invasive species percentage coverages, we utilized one-way ANOVA analysis.

Species Richness. Species richness is defined as the number of distinct species found in an area; for this analysis, each specific species was detailed for a total number of species encountered per site. This analysis includes all species found at a site, and makes no differentiation between native, non-native and invasive species. As this data failed normality testing, it was log-transformed to fit this criteria before testing for significance using one-way ANOVA.

Invasive and Non-native Species. Differing invasive species were found in each prairie, however, this analysis uses eight of the most encountered species to determine presence/absence and whether there is a significant difference between their presence and prairie site history. The eight species chosen were as follows: *Phalaris arundinacea* (reed canary grass), *Bromus inermis* (smooth brome), *Rhamnus cathartica* (common buckthorn), *Melilotus officinale* (yellow sweet clover), *Melilotus alba* (white sweet clover), *Lotus corniculatus* (birds-foot trefoil), *Cirsium arvense* (Canada thistle), and *Berteroa incana* (hoary alyssum). After the species were identified, they were given a binary classification per site (1=present, 0=absent) to be used in contingency analysis for each species, via the Pearson's chi-squared test. This determined whether any of the species were more likely to be found on remnant or restored sites.

A follow-up analysis was utilized to see if the coverage percentages (defined as the mid-point percentage, as detailed by Bohnen & Galatowitsch [2016]) for each of the above-named species had a significant difference in relation to other variables. These midpoints were chosen to be a reliable approximation of the cover percentage of a species seen during surveying. These coverage percentages were analyzed with site history, species richness, area of the sites, and the three management histories, using one-way ANOVA analysis.

Area of Site. Surveyed sites had differing acreage, which was then converted to hectares and compared with other variables to determine potential correlations (e.g. between area of the site and CISA and %PNV scores). This data set failed normality testing, and so was log-transformed for analysis before one-way ANOVA analysis.

The Nature Conservancy Scores. To better consider the land surrounding the prairies that were surveyed, a tool created by the Nature Conservancy was utilized (https://maps.tnc.org/resilientland/), the Resilient Land Mapping Tool. This tool provides a series of scores for determined areas, through the use of polygon-sketching surveyed areas. Three scores are detailed: "Resilience", "Landscape Diversity", and "Local Connectedness" (Anderson et al., 2016). 1) Resilience scores detail an approximate capacity to withstand changes in climate over time, including retaining species diversity and necessary ecological functions (Anderson et al., 2016). Moreover, they were created using elevation data, as well as wetland and soil properties to assess gradients within measured landscapes (Anderson & Barnett et al., 2016). 2) Landscape Diversity scores denote microhabitats in the vicinity of the surveyed area, and any close-proximity gradients (Anderson et al., 2016). These scores were created by accounting for the variety of landforms, elevation range, as well as density and configuration of any wetlands within a 100-acre buffer around each mapped point (Anderson & Barnett et al., 2016). Finally, 3) Local Connectedness scores refer to the degree to which the surveyed area and surrounding landscape are fragmented (Anderson et al., 2016).

These scores denote the natural land cover, compared with human-centric fragmentation caused by major roads, developments, and agricultural lands (Anderson & Barnett et al., 2016). All scores are reported in z-units, and refer to standard deviations above or below the mean, where the mean is detailed to be an average of sites with similar conditions in the ecoregion (Anderson et al., 2016). These metrics were tested against site history via one-way ANOVA, to see if remnant or restored prairies scored significantly higher, and if so, which metric(s) corresponded to this pattern.

August Follow-up Surveys. Surveys conducted during August 2019 provided an opportunity for comparison between two distinct time frames within the same growing season. These surveys utilized the same surveying protocol, and consisted of a subset of 11 of the original 31 prairies (Table 2). The returned CISA and %PNV scores were compared to the earlier season scores using a paired t-test, to determine if there was a significant difference between the June/July and the August sampling period scores for the two stated metrics. As well, the species richness between this subset was compared to the same subset in the earlier season findings, using a paired t-test.

Phylogenic Diversity. We utilized one-way ANOVA analysis to test for significant relationships with site ownership, site area, frequency of each of the three detailed management types, as well as years since the last occurrence of each management type, CISA and %PNV score, species richness for both June/July and August, as well as the three Nature Conservancy (TNC) scores. We also tested for interactions between significant results and phylogenetic diversity via two-way ANOVA.

RESULTS

CISA and %PNV Scores.

Following a 31-site survey (Figure 1), we saw a marked increase in %PNV score for restored prairies ($F_{(1,30)}$=4.8146, p=0.0364; Figure 3), though there was not a significant difference between CISA score and site history (restored and remnant prairies), $F_{(1,30)}=0.0026$, $p=0.9596$. For both June/July surveys, and August surveys the corresponding Bohnen & Galatowitsch quality figures were made (Figure 4) which illustrate individual site quality relative to other surveyed sites. We also did not see a significant difference between the area of a site and its corresponding CISA or %PNV scores; $F_{(1,30)}=0.0103$, $p=0.9198$ and $F_{(1,30)}=0.5854$, $p=0.4504$, respectively.

Site History.

Site history was also compared to species richness, frequency of management and invasive species presence. Comparing site history to species richness, we found that there was no appreciable change in species richness between remnant and restored prairies ($F_{(1,30)}=0.7240$, $p=0.4018$).

We also tested whether management choices were significantly different between the two site history conditions. We did not see a significant result for fire as a management strategy, $F_{(1,30)}=0.0240$, $p=0.8779$, or mechanical management, $F_{(1,30)}=0.5131$, $p=0.4795$, or chemical management, $F_{(1,30)}=1.0877$, $p=0.3056$. From this, we find that there does not appear to be a significant relationship between which type of management was chosen for the two site histories, and the management regimes between both site histories were comparable.

Management History.

The multiple regression analysis between management history and CISA score indicated that something other than the three selected management histories was acting upon the invasive species seen at each of the sites. As such, $F_{(2,29)}=1.3068$, $p=0.617$, indicating that the three described variables are not accounting for variation within the CISA score observed between the sites, and that the results are not significant. Additionally, frequency of mechanical management ($p=0.34905$) did not show a significant result. Neither did the other two variables, chemical management frequency ($p=0.35129$) and fire frequency ($p=0.54669$).

Regarding %PNV scores, the multiple regression also indicated no significant relationships between the variables ($R^2=0.06$); $F_{(2,29)}=4.5308$, $p=0.6274$. Moreover, fire frequency ($p=0.20418$), frequency of mechanical management ($p=0.62654$), and frequency of chemical management ($p=0.72726$) also indicate this.

We tested to see whether management history (both frequency of occurrence [2009-2019], as well as years since [2009-2019] each management type was applied) varied significantly between restored and remnant prairies. Regarding the years since each type, no significant differences were found for burning ($F_{(1,30)}=0.7401$, $p=0.3998$), mechanical management ($F_{(1,30)}=0.0378$, $p=0.8471$) or for chemical management ($F_{(1,30)}=1.5980$, $p=0.2163$). Comparably, there were also no significant differences for frequency of burns ($F_{(1,30)}=0.0240$, $p=0.8779$), frequency of mechanical management ($F_{(1,30)}=0.5131$, $p=0.4795$), or frequency of chemical management ($F_{(1,30)}=1.0877$, $p=0.3056$). Following this, we also tested whether there were any significant differences associated with public versus private prairies, and several variables (CISA score, %PNV score, and species richness). We found no significant difference in the CISA score ($F_{(1,30)}=0.2541$, $p=0.6810$) or %PNV score ($F_{(1,30)}=0.0009$, $p=0.9758$). Finally, with regard to species richness, we see that this is also unaffected ($F_{(1,30)}=0.8405$, $p=0.3668$).

In order to determine whether there were any differences in CISA score, %PNV score and species richness tied to ownership in the August surveys, we found that CISA score and ownership indicated no significant difference ($F_{(1,10)}=0.9815$, $p=0.3477$). For %PNV score, we do see a trend towards significance, as $F_{(1,10)}=3.4014$, $p=0.0982$, where public prairies had a mean increase of 24.24% compared to private ones (Figure 5). And finally, with species richness, we see that $F_{(1,10)}=3.1557$, $p=0.1094$, which is not significantly different. The results for the number of years since the last management type application and frequency were predominately similar and lack significance (Appendix; Tables 1 and 2), but this analysis did return some significant relationships (Figure 6).

Invasive and Non-native Species.

In order to test whether there was a difference in invasive and non-native species presence between the two site histories, a contingency analysis was done for each of the eight individual species of interest (Table 3); *Bromus inermis* was found at all sites, so chi-squared was not applied for this species. No significant difference in frequencies were returned between any of the invasive and non-native species, and site history (Table 3).

Regarding site history and each of the eight species' coverage percentages, no significant relationships were detailed. For *Phalaris arundinacea* $F_{(1,30)}=1.0631$, $p=0.3110$; for *Bromus inermis* $F_{(1,30)}=0.6754$, 0.4179; for *Rhamnus cathartica* $F_{(1,30)}=2.0820$, $p=0.1598$; for *Melilotus officinalis* $F_{(1,30)}=0.7439$, $p=0.3955$; for *Melilotus alba* $F_{(1,30)}=1.5388$, $p=0.2247$; for *Lotus corniculatus* $F_{(1,30)}=1.3783$, $p=0.2499$; for

Cirsium arvense $F_{(1,30)}$=0.4051, p=0.5295; and finally, for *Berteroa incana*, $F_{(1,30)}$=0.1325, p=0.7185.

Similarly, we found no significant relationships between June/July species richness and invasive species coverage percentages. For *Phalaris arundinacea* $F_{(1,30)}$=2.1409, p=0.1542; for *Bromus inermis* $F_{(1,30)}$=0.6655, p=0.4213; for *Rhamnus cathartica* $F_{(1,30)}$=1.3439, p=0.2558; for *Melilotus officinalis* $F_{(1,30)}$=2.7625, p=0.1073; for *Melilotus alba* $F_{(1,30)}$=0.1250, p=0.7263; for *Lotus corniculatus* $F_{(1,30)}$=0.0644, p=0.8015; for *Cirsium arvense* $F_{(1,30)}$=1.0771, p=0.3079; and finally, for *Berteroa incana*, $F_{(1,30)}$=0.1702, p=0.6830. However, we did find a significant result between August species richness and invasive species coverage percentages; *Berteroa incana* coverage percentages decreased significantly ($F_{(1,10)}$=9.2204, p=0.0141) with increasing species richness (Figure 7). For *Phalaris arundinacea* $F_{(1,10)}$=0.0259, p=0.8756; for *Bromus inermis* $F_{(1,10)}$=1.8711, p=0.2045; for *Rhamnus cathartica* $F_{(1,10)}$=0.0234, p=0.8817; for *Melilotus officinalis* $F_{(1,10)}$=0.6193, p=0.4515; for *Melilotus alba* $F_{(1,10)}$=0.3475, p=0.5700; for *Lotus corniculatus* $F_{(1,10)}$=0.0000, p=0.9993; for *Cirsium arvense* $F_{(1,10)}$=0.18406, p=0.2079.

Regarding the area of the site and each of the eight species' coverage percentages, we see some interesting results with *Melilotus officinalis*: $F_{(1,30)}$=12.7399, p=0.0013, where increasing the area of a site significantly increased this species' coverage (Figure 8). We found no other significant correlations with the other tested species: for *Phalaris arundinacea* $F_{(1,30)}$=0.0032, p=0.3272; for *Bromus inermis* $F_{(1,30)}$=0.1046, p=0.7487; for *Rhamnus cathartica* $F_{(1,30)}$=0.0668, p=0.7979; for *Melilotus officinalis* $F_{(1,30)}$=12.7399,

$p=0.0013$; for *Melilotus alba* $F_{(1,30)}=0.0155$, $p=0.9016$; for *Lotus corniculatus* $F_{(1,30)}=0.0936$, $p=0.7619$; for *Cirsium arvense* $F_{(1,30)}=3.6551$, $p=0.0658$; and finally, for *Berteroa incana*, $F_{(1,30)}=0.8200$, $p=0.3727$.

The Nature Conservancy (TNC) Scores.

In comparing the TNC scores, the major consideration was whether there was a significant difference in their ratings between restored and remnant sites, and we did see significant differences (Figure 9). We found that the mean difference for Resilience scores was 245.8% higher for restorations than for remnants; similarly, the mean restoration scores for Landscape Diversity (213.9%) and the mean scores for Local Connectedness (303.4%) were also significantly higher than remnant sites. For Resilience, $F_{(1,30)}=4.3670$, $p=0.0455$, where restored sites had significantly higher Resilience scores than remnant sites. Regarding Landscape Diversity, $F_{(1,30)}=4.1591$, $p=0.0506$, where again restored sites scored higher on the given metric than the remnant sites. Finally, to address Local Connectedness, $F_{(1,30)}=4.2571$, $p=0.0481$.

August Follow-up Surveys.

In comparing the subset of 11 prairies surveyed in August, to the 11 matched prairie surveys from June/July, the outcome indicates that there was not a significant difference between the two CISA conditions: June/July (22.16 ± 3.86) and August (23.18 ± 4.07); $t_{(20)}=0.33$, $p=0.75$. However, we did find a significant difference between the two %PNV conditions. We found that for June/July surveys (22.63 ± 1.43) and the August surveys (42.98 ± 2.82), there were more native species identified during the August surveys ($t_{(20)}=5.24$, $p<0.0001$). Moreover, when comparing June/July (52.45 ±

4.32) and August (69.55 ± 4.9), we found a significant difference where there was 32.59% greater species richness in August than in the June/July period; $t_{(20)}=3.04$, $p=0.0125$ (Figure 10).

Phylogenic Distance.

Phylogeny (Figure 2) values returned both significant and non-significant results. First, we did see a significant relationship, where increasing June/July species richness ($F_{(1,30)}=111.4380$, $p<0.0001$) led to higher phylogenetic diversity values; moreover, August species richness also trended towards a similar positive relationship ($F_{(1,30)}=3.4699$, $p=0.0954$; Figure 11). Additionally, phylogenetic diversity was not due to invasive species (CISA score [$F_{(1,30)}=0.0392$, $p=0.8445$]) or %PNV score ($F_{(1,30)}=0.0568$, $p=0.8133$; Figure 12). We also found no significant difference between restored and remnant prairies' phylogenetic diversity ($F_{(1,30)}=1.0200$, $p=0.3209$, Figure 13), nor for site ownership (public versus private, [$F_{(1,30)}=0.9274$, $p=0.3435$]).

However, we did find that phylogenetic diversity (Code 2, 374 total species) increased significantly with increasing site area ($F_{(1,30)}=21.4173$, $p<0.0001$; Figure 14), and higher frequency of fire led to larger phylogenetic distance values ($F_{(1,30)}=5.1646$, $p=0.0306$; Figure 14). Moreover, we also found a trend towards significance with TNC Local Connectedness scores ($F_{(1,30)}=3.2423$, $p=0.0822$; Figure 14). We did not see any significant relationships between phylogenetic diversity and frequency of mechanical or chemical management, the years since any of the three management categories, or TNC Landscape Diversity or Resilience scores (Table 4). We did not see any significant results in our two-way analyses, which also tested for interactions between the two variables: site ownership and site history returned $F_{(2,30)}$=1.9813, p=0.1567. We saw a similar lack of main effect interaction with frequency of fire (since 2009) and site history: $F_{(2,30)}$=2.0827, p=0.1405.

DISCUSSION

Across 31 sites in Southern Minnesota (Figure 1), we found that restored prairies scored significantly higher than remnants in %PNV score (Figure 2), however, we were surprised to see that CISA did not differ significantly, which is not what we might expect from literature (Hillhouse et al., 2011; Corpstein et al., 2014). Moreover, the area of the prairies did not have any effect on either of these scores, which may be expected from existing research regarding native and invasive species richness (Cully et al., 2003). We also found that management strategy did not vary significantly between public and private ownership; both sectors are utilizing similar strategies, and at similar intervals and frequencies. This is an encouraging finding, in that, while remnants may not be able to reach the %PNV of the restored sites, they appear to have been able to keep invasive species from adversely affecting their biodiversity.

Curiously, while we did not find significant differences in which invasive species are found in remnant and restored prairies, we did find that some species can be affected by management strategies, which is supported by other findings (Stanley et al., 2008). For instance, we found that *Melilotus alba* coverage increased significantly the longer that a site had gone without mechanical management and chemical management (Figure 6). *Melilotus officinalis* coverage also increased significantly on prairies with a larger area (Figure 8). Moreover, *Berteroa incana* coverage percentages decreased significantly with increasing species richness in the June/July sampling period (Figure 7). However, both of the latter two analyses appear to have significant results due to a possible outlier; further surveys of similar large sites are necessary to elucidate whether these patterns are representative. Additionally, none of the other species we tested showed comparable results. Based on these findings, we concur with Larson et al. (2001); undesirable species invasion is often highly uncertain, and that the type of vegetation is an important variable to consider in management.

Prairie vegetation species richness was also of great interest to us, because it is used as a metric so extensively in research as a metric for site quality and community resilience (Larson, 2002; Larson et al., 2011; Corpstein et al., 2014; Millikin et al., 2016; Heslinga et al., 2010). Timing of the survey was important-we found that our initial June/July survey period had lower species richness when compared to the later August subset surveys. Specifically, we found that August surveys had 32.59% greater species richness than the earlier June/July surveys; we also saw more native species that were identified during those surveys (Figure 4). We can infer from these findings that one iteration of the survey metric is likely insufficient to fully capture a representative snapshot of the plant community of these prairies; follow-ups are necessary. Additionally, it may not be plastic enough for seasonal shifts and irregularities, like we encountered during the unusually cool, wet start to the 2019 growing season.

Moreover, when we tested remnant and restored prairies' respective scores on the three detailed TNC metrics, we saw that, on average, restorations actually scored significantly higher in all metrics (resilience, landscape diversity and local connectedness, [Figure 9]). These are important findings: first, the resilience scores were defined as indicating the potential for any given studied area to withstand change, retain biodiversity and retain critical ecological functions (Anderson et al., 2016). We found that Resilience was 245.8% higher for restorations; this implies that these landscapes have greater potential to withstand shifts as the landscape continues through time. Moreover, they have a greater potential to retain their characteristic biodiversity, and to provide key ecological functions, which will doubtless become increasingly important over time. Second, Landscape Diversity was 213.9% higher for restorations, a score that reflects all nearby microhabitats, as well as any climatic gradients (Anderston et al., 2016). It follows that restorations span a more diverse set of topographic and microclimate conditions, because restorationists have been creating them wherever possible. It is concerning however that, because remnant sites scored significantly lower, they are by extension at greater risk of adverse effects cause by that lack of diversity. Finally, Local Connectedness was 303.4% higher for restorations. This score reflects the degree to which the prairie and surrounding natural areas are fragmented (Anderson et al., 2016). It has long been established that fragmentation is an undesirable state for natural areas (Leimu et al., 2010) and that more connected landscapes have greater potential for resilience and conservation (Belote et al., 2017).

One of our most significant analyses in this study was the phylogenetic diversity analysis, and how it connected to management strategy, prairie area and fragmentation. Restorations are relatively rarely assessed for phylogenetic diversity (Barber et al., 2017), despite it being a versatile, important metric for community composition. Our results indicated that June/July species richness was strongly correlated with increased phylogenetic diversity, which is further supported by a trend towards significance in with August species richness (Figure 11). Additionally, there was no correlation with CISA score, indicating that the diversity we describe is not a function of invasive species presence (Figure 12). Curiously, we did not find a significant difference between phylogenetic diversity and site history (Figure 13). Site ownership also did not have a significant relationship, indicating that both private and public entities are able to foster comparable phylogenetic diversity in their prairies. However, there was a trend towards significance during the August survey period where public prairies scored 24.24% higher in %PNV than private prairies (Figure 5). We also found that phylogenetic diversity had a significant positive relationship with increasing site area, and a trend towards significance in a positive correlation with TNC Local Connectedness scores (Figure 14). This indicates that larger sites, with more possibility for connection with other natural areas are able to either retain or promote increased phylogenetic diversity. This is also what we would expect to see based on existing context provided by fragmentation (Leimu et al., 2010).

Perhaps our most significant finding was a strong correlation between increasing prescribed burn frequency and phylogenetic diversity (Figure 14); curiously, we did not see any correlations between phylogenetic diversity and either mechanical or chemical management. However, research has long established the importance of prescribed burns on the prairie ecosystem and it’s ties to the plant community (Vinton et al., 1993; Brye et al., 2002; Schmithals et al., 2014; Kricsfalusy & Esparrago, 2015; Winter et al., 2015). One should acknowledge there would be a limit to the positive effect of fire disturbance frequency (Intermediate Disturbance Hypothesis; Connell, 1978), and acknowledge that too much prescribed burning will harm the plant community. However, we do see in this study that phylogenetic diversity was increased significantly with higher prescribed burn frequency. Furthermore, we found that phylogenetic diversity was not correlated with our invasive species metric (CISA score), but was strongly correlated with species richness for both June/July and displayed a positive trend towards significance August. This indicates that phylogenetic diversity can potentially serve as a metric, to aid managers in assessing site quality.

Overall, with the exception of burn frequency, site management histories did not elucidate much of the variation we detailed in this study. It was difficult to obtain detailed management records, and to compare different record styles to each other. It is likely that fine detail necessary to find these outcomes was lost in the broad-strokes approach necessity dictated for this analysis. Moreover, other considerations like seed mix origin and richness, soil nutrient content, soil invertebrate and microbial community, among many other variables were not included in this analysis, and would likely provide illuminating context. We also noticed that the MN DNR Native Plant Community lists do not include all the native species found; rather, a representative sample (MN DNR 2013; Bohnen & Galatowitsch, 2016). Including all necessary data for the most accurate assessments would necessarily require the prioritization of resources for frequent, indepth monitoring over extended periods of time; this level of detail is likely not feasible for the majority of management sites.

CONCLUSIONS

The results of our study support that timing and frequency of plant surveys are critical considerations; we found greater species richness in our later-season follow-up surveys than in our original, earlier-season surveys. Moreover, we found more native species during the follow-up surveys. This combination implies that one survey is insufficient to capture much of the diversity found in Southern Minnesotan prairies; when the surveys are done (June/July or later, during August) and how often they are done (one survey only, or multiple site visits over the duration of the growing season) are important considerations in order to limit omission and an inaccurate depiction of the community. By extension, this snapshot-like approach may under-rate site quality, and appears generally insufficient.

Interestingly, we also found during both surveys (June/July and August) that restored prairies scored significantly higher in the %PNV metric than remnant prairies, though the CISA metric did not illustrate a comparable pattern. Additionally, we found that type, and timing, of management on these sites can be important: we found a strong positive correlation between increasing prescribed burn frequency and phylogenetic diversity, though we did not see similar patterns for either mechanical or chemical management. Based on this result from our study, we conclude that prescribed burning is incredibly important for enhancing native species richness, and phylogenetic diversity in these prairies and other management techniques do not provide the same benefit. However, we did find it difficult to find a single cause, or a single preventative measure, for invasive species presence on different sites.

We did not find many significant differences between remnant/restored prairies in terms of site quality, though the Nature Conservancy metrics did illustrate significant differences between the two. Based on these results, the Nature Conservancy metrics have significant potential as tools to select for sites in the future. Overall, our study illustrates the necessity of monitoring plant biodiversity and cover in both remnant and restored prairies, and provides an argument for increased surveying over multiple growing seasons to inform management approaches for site quality improvement.

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FIGURE LEGENDS

Figure 1. Map of all survey locations; many locations included multiple prairies.

Figure 2. Fan-type phylogenetic tree of the master phylogeny; 374 total species, pruned down from the Smith and Brown (2018) phylogeny.

Figure 3. Mean %PNV scores were 34.48% higher for restored sites than for remnant sites. Error bars are ±1 standard error.

Figure 4. Percent Native Vegetation Scores (%PNV) and Composite Invasive Species Scores (CISA) for surveyed prairies during the June/July survey period (N=31) and the August follow-up (N=11). The median lines for June/July are as follows: %PNV=22.727, and CISA=19. Similarly, for August the median lines are as follows: %PNV=43.55, and CISA=15. This separates into quadrants denoting quality: upper left quadrant denotes low quality, upper right and lower left denote medium quality, and lower right denotes high quality.

Figure 5. Public prairies had a mean increase of 24.24% in %PNV score when compared to private prairies during the August sampling period. Error bars are ±1 standard error.

Figure 6. *Melilotus alba* coverage percentages increased significantly the longer that prairies had gone without mechanical (black) or chemical (red) management ($p=0.0485$, and $p=0.0133$, respectively).

Figure 7. The August sampling of *Berteroa incana* coverage percentages decreased significantly ($p=0.0141$) as species richness increased.

Figure 8. Coverage percentages for *Melilotus officinalis* increased significantly ($p=0.0013$) with increasing site area.

Figure 9. Nature Conservancy mean metric scores for remnant and restored prairies, with error bars ± 1 standard error. For all three metrics, restorations scored significantly higher than remnants.

Figure 10. Species richness at all surveyed prairies for the June/July surveys ($N=31$) and for the August follow-up surveys ($N=11$).

Figure 11. Phylogenetic distance values compared with species richness for both the June/July (black) and August (red) surveys. Larger phylogenetic distance is strongly correlated ($p<0.001$) with higher June/July species richness, and there is a similar positive trend towards significance with August species richness ($p=0.0954$).

Figure 12. Phylogenetic diversity scores compared to %PNV and CISA score: neither metric illustrated a significant correlation.

Figure 13. Mean phylogenetic diversity score for remnant and restored prairies; error bars are ± 1 standard error. We found no significant difference between remnant and restored prairies' phylogenetic diversity.

Figure 14. Significant relationships between phylogenetic diversity and Frequency of Fire (since 2009), TNC Local Connectedness metric and area of the site (in hectares). All three variables indicate a positive correlation with phylogenetic diversity.

TABLE LEGENDS

Table 1. Surveyed prairies and their respective composite invasive species scores (CISA) and percent native vegetation scores (%PNV), as well as ownership (The Nature Conservancy [TNC], Minnesota Department of Natural Resources [MN DNR] or Privately Owned [Private]). All sites not in public domain are labelled private for security; sites with >1 surveyed prairie are indicated with a representative letter and number.

Table 2. August 2019, subset (N=11) of originally surveyed prairies (N=31) and their respective composite invasive species scores (CISA) and percent native vegetation scores (%PNV).

Table 3. Invasive species analysis for species found at all June/July sites, showing Pearson's chi-squared test results.

Table 4. One-way ANOVA results for phylogenetic diversity and assorted variables. None of which indicate significant correlations.

FIGURES AND TABLES

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Prairie	Ownership	Composite Invasive Species Abundance	% Native Vegetation
Afton State Park	MN DNR	5	30
Antelope Valley SNA (AV-1)	MN DNR	5	22.7
Antelope Valley SNA (AV-2)	MN DNR	50	19.4
Blaine Preserve SNA	MN DNR	27	17.9
Blue Devil Valley SNA	MN DNR	93	4.5
Butternut Valley Prairie SNA	MN DNR	9	28.2
Compass Prairie SNA	MN DNR	22	33.3
Cottonwood River Prairie SNA	MN DNR	55.5	20.8
Flandrau State Park	MN DNR	19.5	14.3
Fort Ridgely State Park (A-1)	MN DNR	7	33.3
Fort Ridgely State Park (A-2)	MN DNR	32.5	29.5
Fort Ridgely State Park (A-3)	MN DNR	32	26.3
Glynn Prairie SNA	MN DNR	3	29.4
Kasota Prairie SNA	MN DNR	19	14.1
Langhei Prairie SNA	MN DNR	1	22
Private 1	Private	43.5	31.7
Oronoco Prairie SNA	MN DNR	26	24.1
Racine Prairie SNA	MN DNR	24.5	7.1
Private 2 (R-1)	Private	1.5	22.7
Private 2 (R-2)	Private	4.5	24.1
Private 2 (R-3)	Private	33.5	18.2
Private 2 (R-4)	Private	30.5	21.9
Private 2 (R-5)	Private	2	32.6
Private 2 (R-6)	Private	2	18.2
River Terrace Prairie SNA	MN DNR	5	10.9
Roscoe Prairie SNA	MN DNR	6.5	36.4
Schaefer Prairie Preserve	TNC	11	22.1
Staffanson Prairie	TNC	10	27.9
Private 3	Private	42.5	12.3
Private 4 (V-1)	Private	7	17.5
Private 4 (V-2)	Private	56.6	27.8

Table 2. August 2019, subset (N=11) of originally surveyed prairies (N=31) and their respective composite invasive species scores (CISA) and percent native vegetation scores (%PNV).

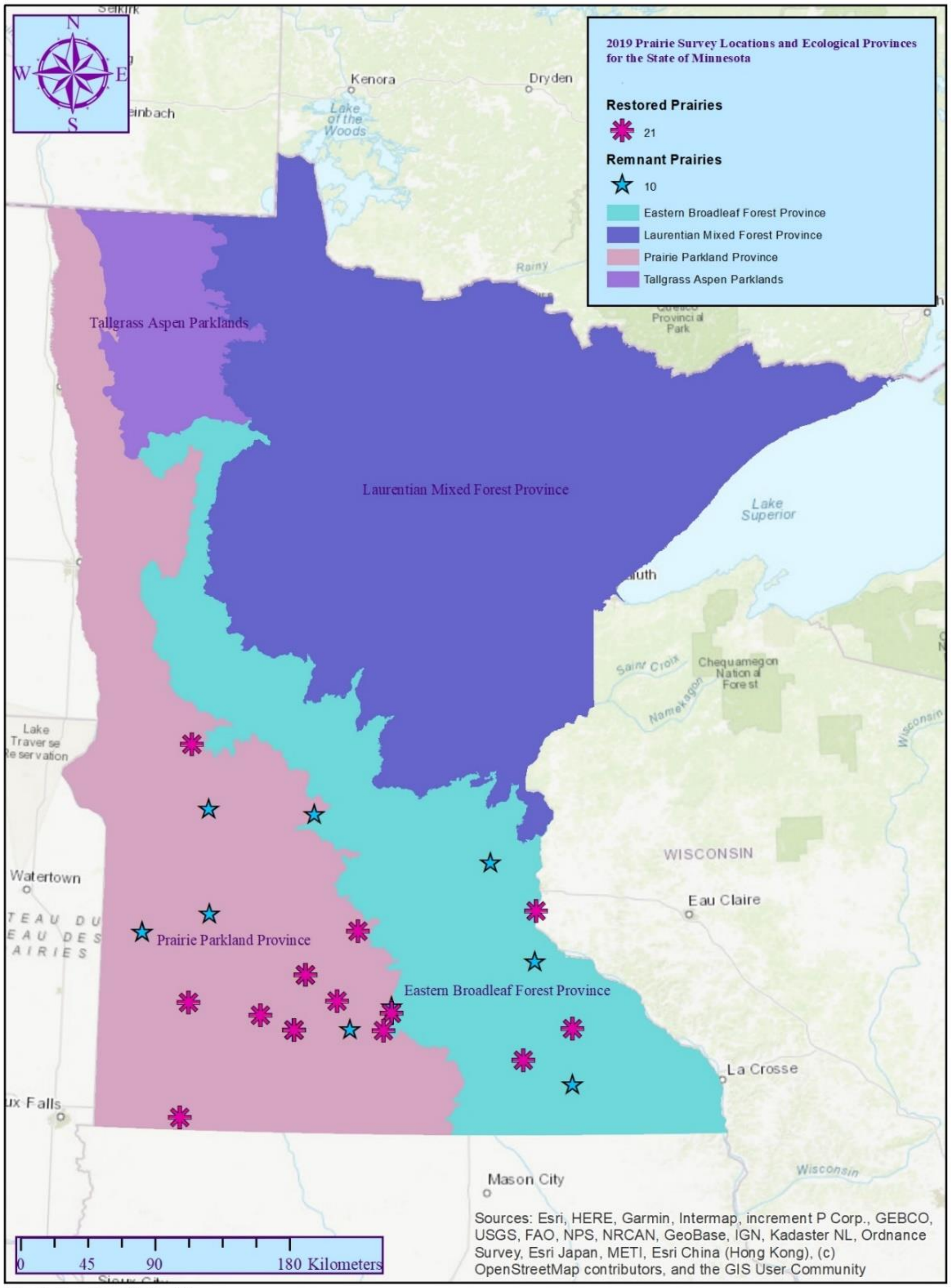
Prairie	Composite Invasive Species Abundance	% Native Vegetation
Butternut Valley SNA	12	55.8
Compass Prairie SNA	45	54.6
Private 1	9	34.4
Kasota Prairie SNA	12	56
Oronoco Prairie SNA	30	41.6
Private 2 (R-1)	9	43.8
Private 2 (R-5)	15	37.3
River Terrace Prairie SNA	39	32.6
Private 3	33	29.2
Private 4 (V-1)	15	44.1
Private 4 (V-2)	36	43.6

Table 3. Invasive species analysis for species found at all June/July sites, showing Pearson's chi-squared test results.

Species Name	Pearson	P
Phalaris arundinacea	0.007	0.9347
Bromus inermis	0	n/a
Rhamnus cathartica	0.194	0.6597
Melilotus officinale	1.312	0.2520
Melilotus alba	2.178	0.1400
Lotus corniculatus	1.015	0.3137
Cirsium arvense	1.072	0.3006
Berteroa incana	0.136	0.7127

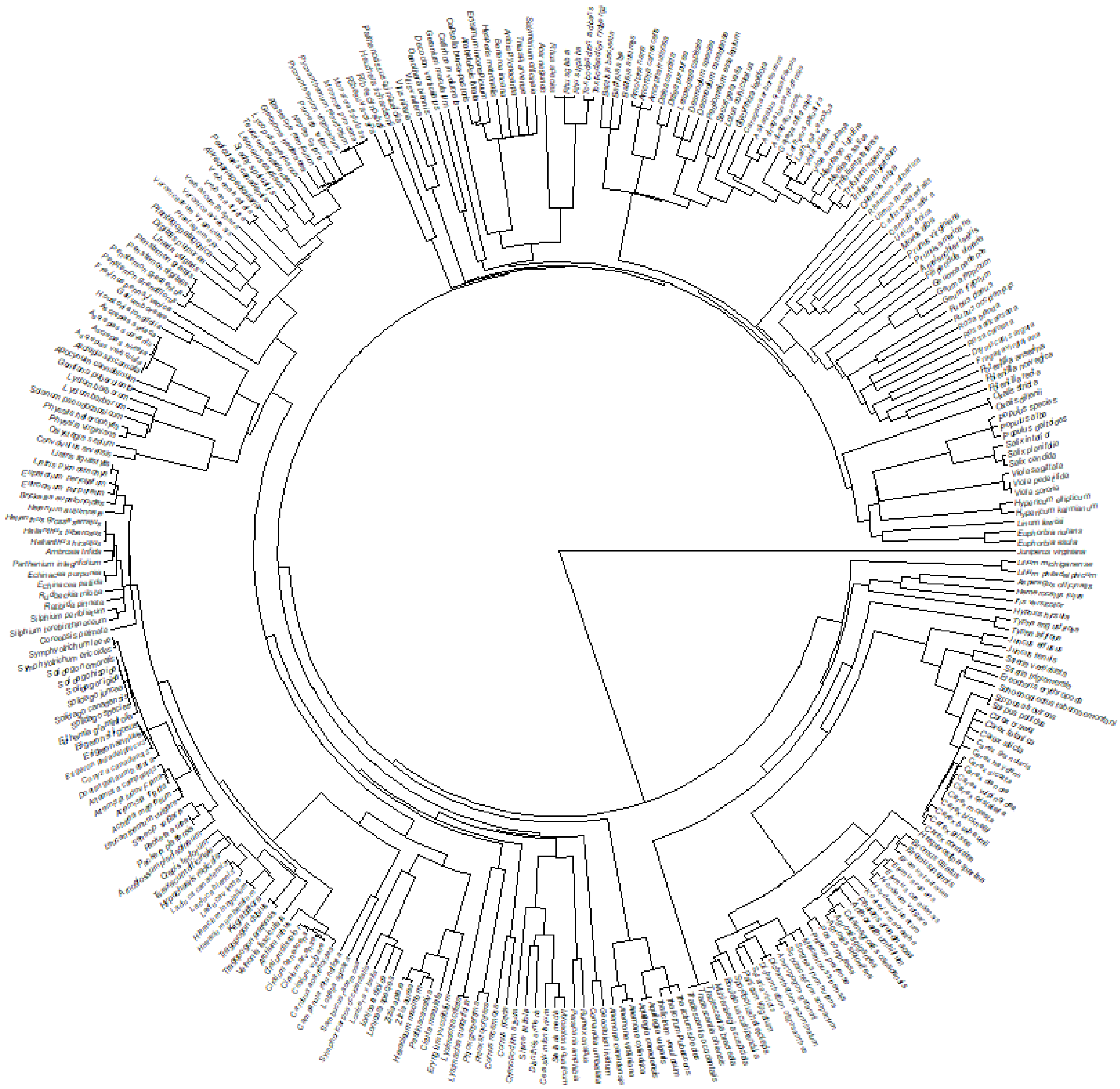
Table 4. One-way ANOVA results for phylogenetic diversity and assorted variables. None of which indicate significant correlations (DF[1, 30]).

One-way ANOVA Analysis of Phylogenetic Diversity and:	F	P
Frequency of mechanical management	0	0.9958
Frequency of chemical management	0.195	0.6624
Years since the last application of burn management	0.928	0.3433
Years since the last mechanical management	0.104	0.749
Years since the last chemical management	0.194	0.6627
TNC Resilience	2.099	0.1581
TNC Landscape Diversity	1.095	0.3039



Map Produced by: Ainsley Peterson
Survey Data: Ainsley Peterson
Basemap: ESRI
Minnesota Ecological Provinces Layer: Minnesota Department of Natural Resources

Figure 1. Map of all survey locations; many locations included multiple prairies.



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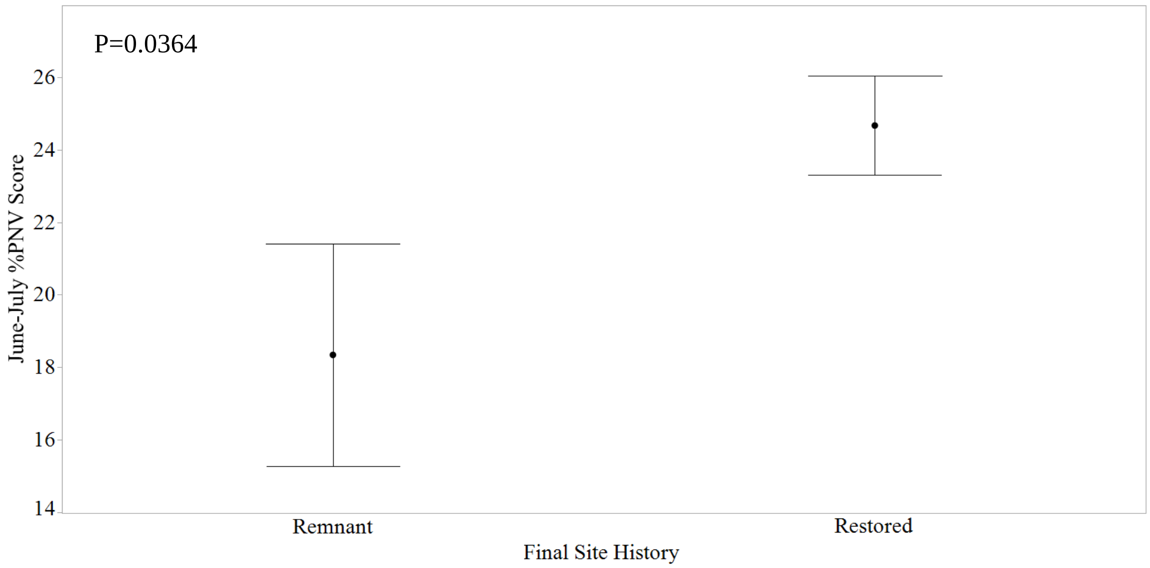

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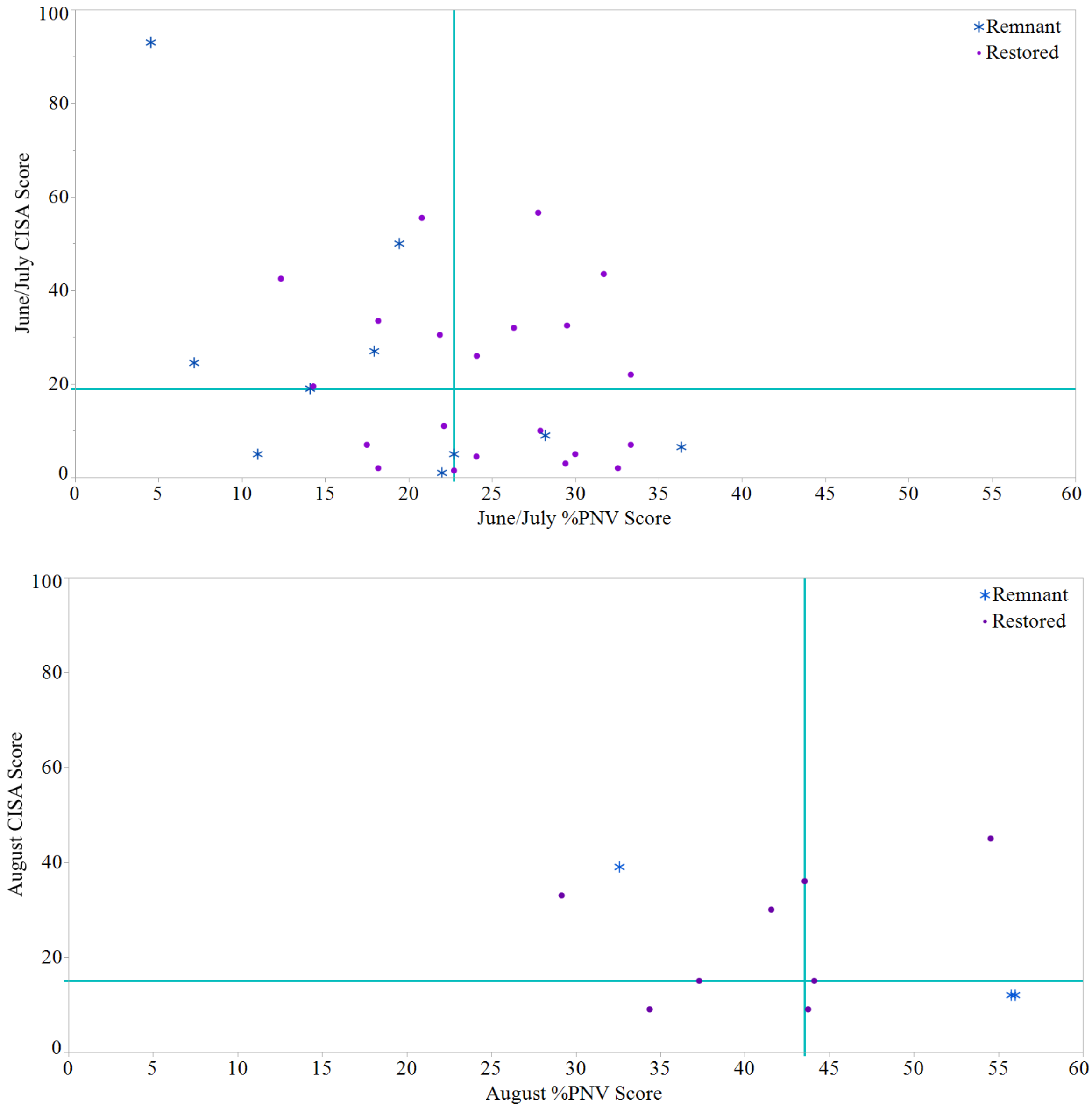

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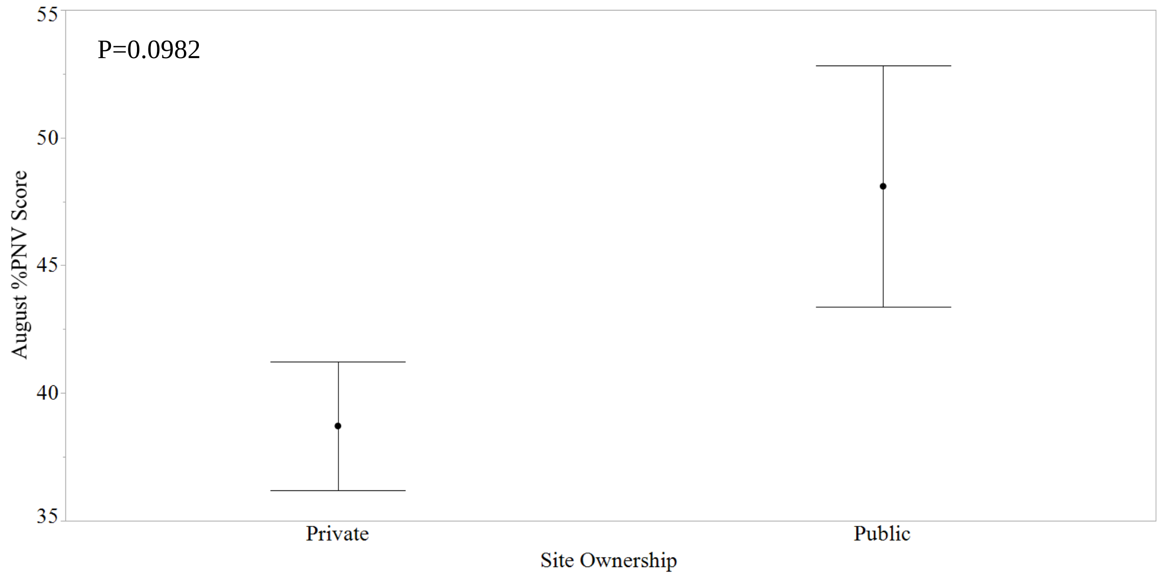

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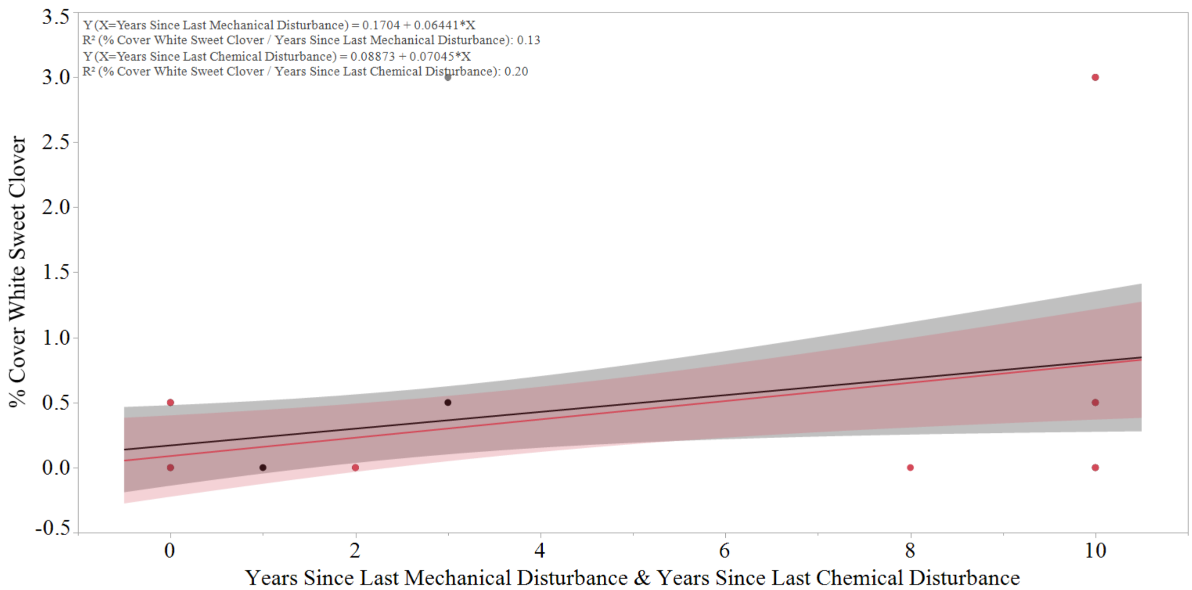

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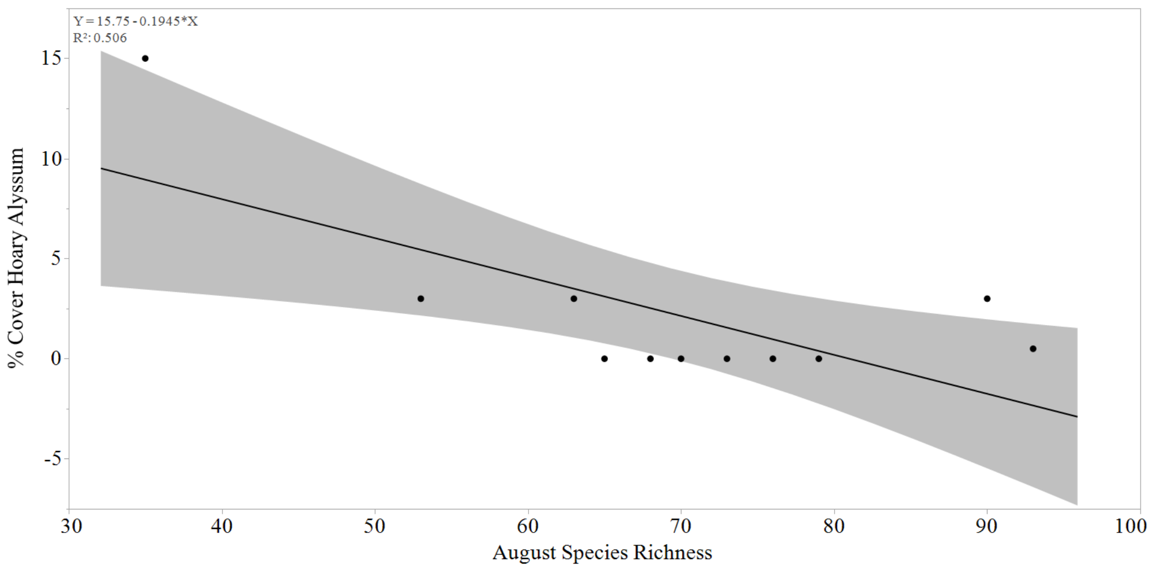

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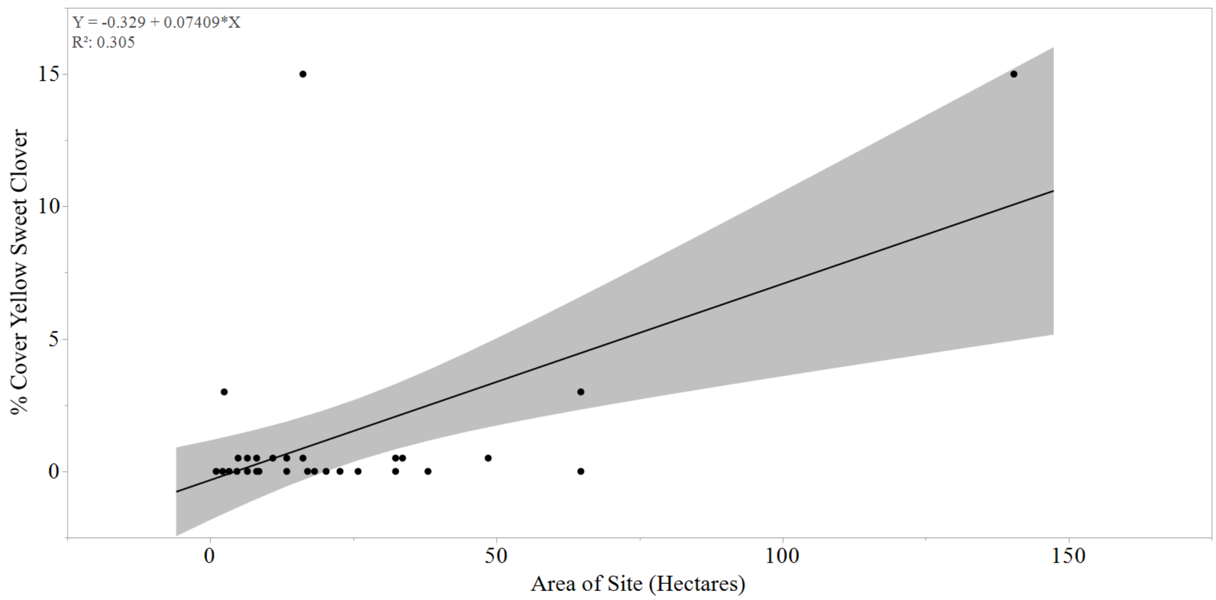

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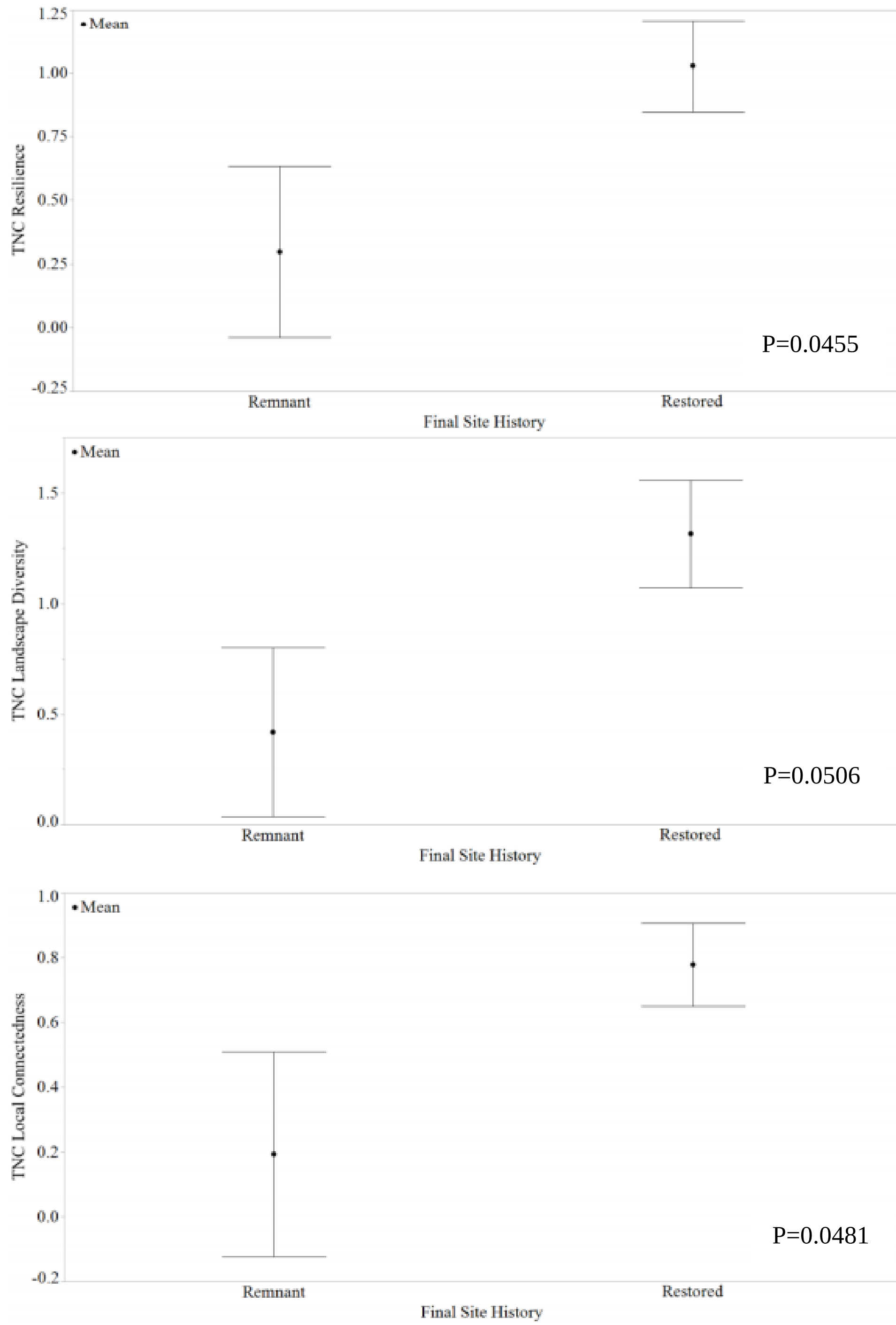

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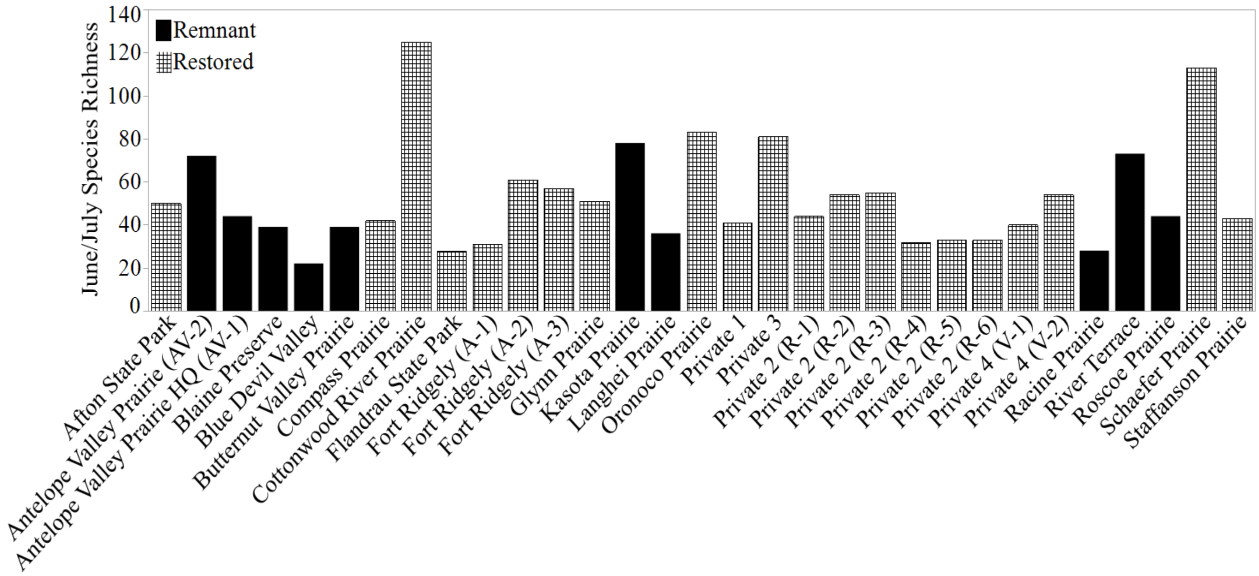

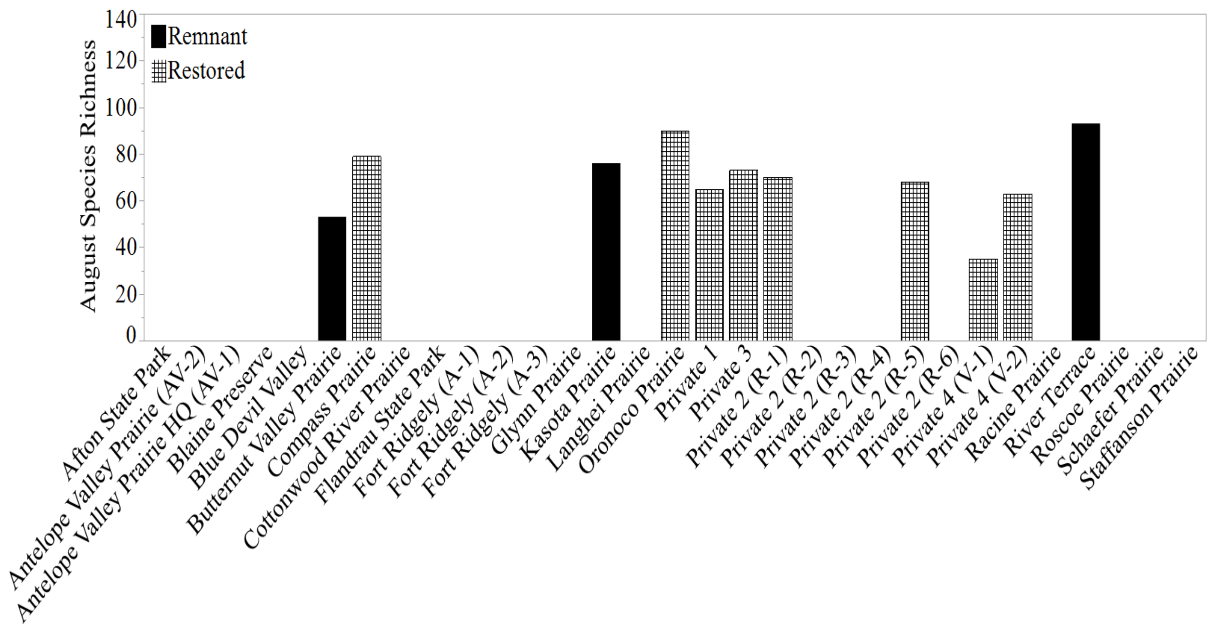

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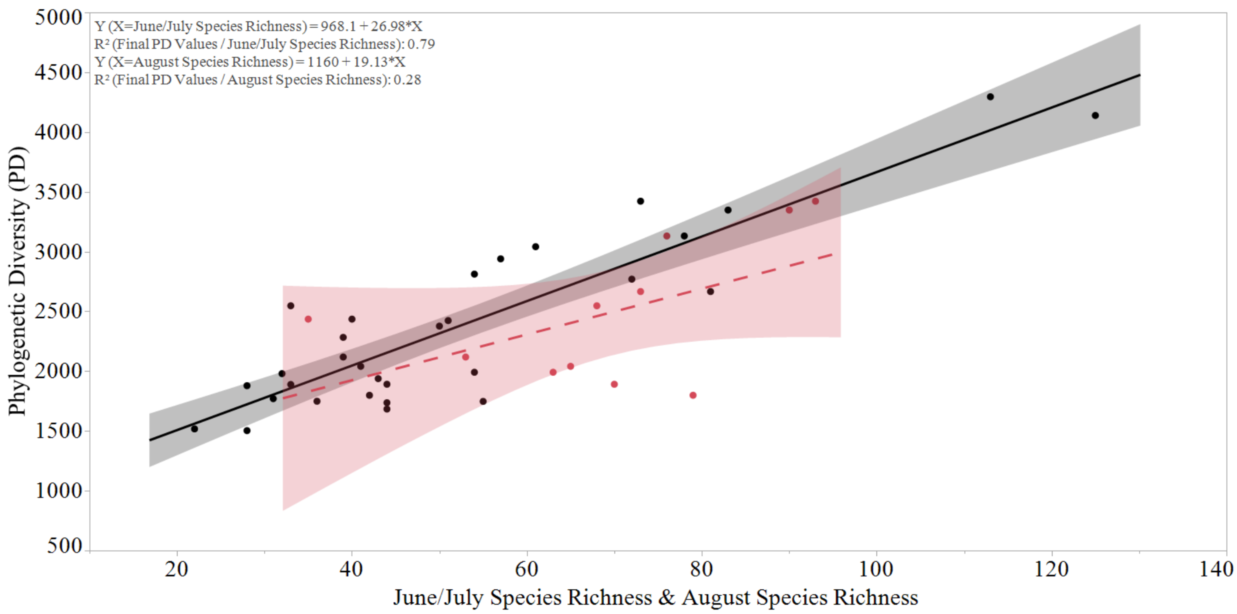

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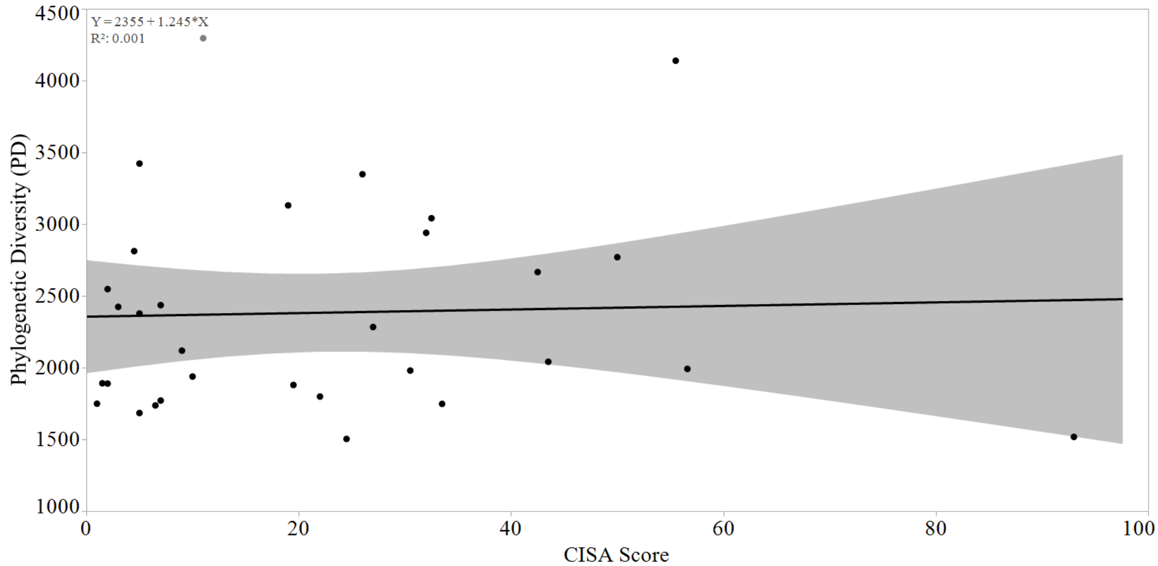

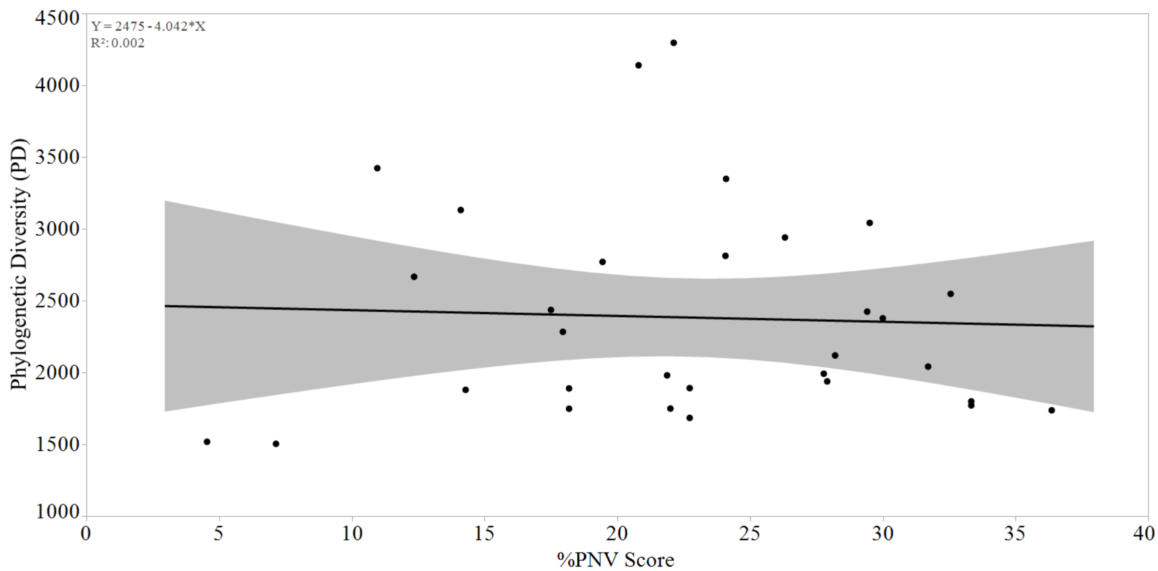

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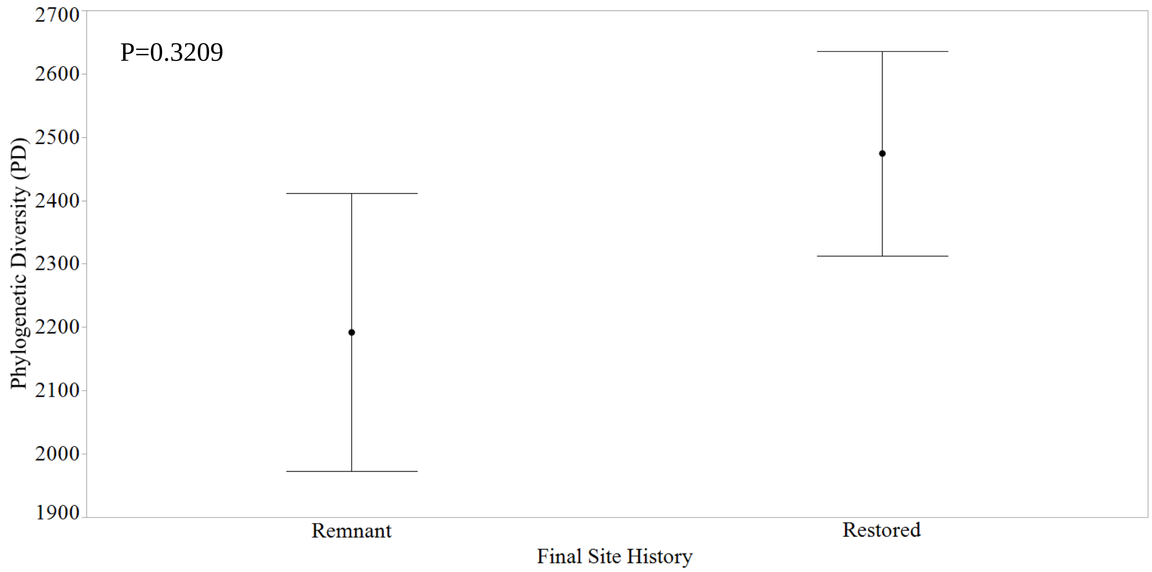

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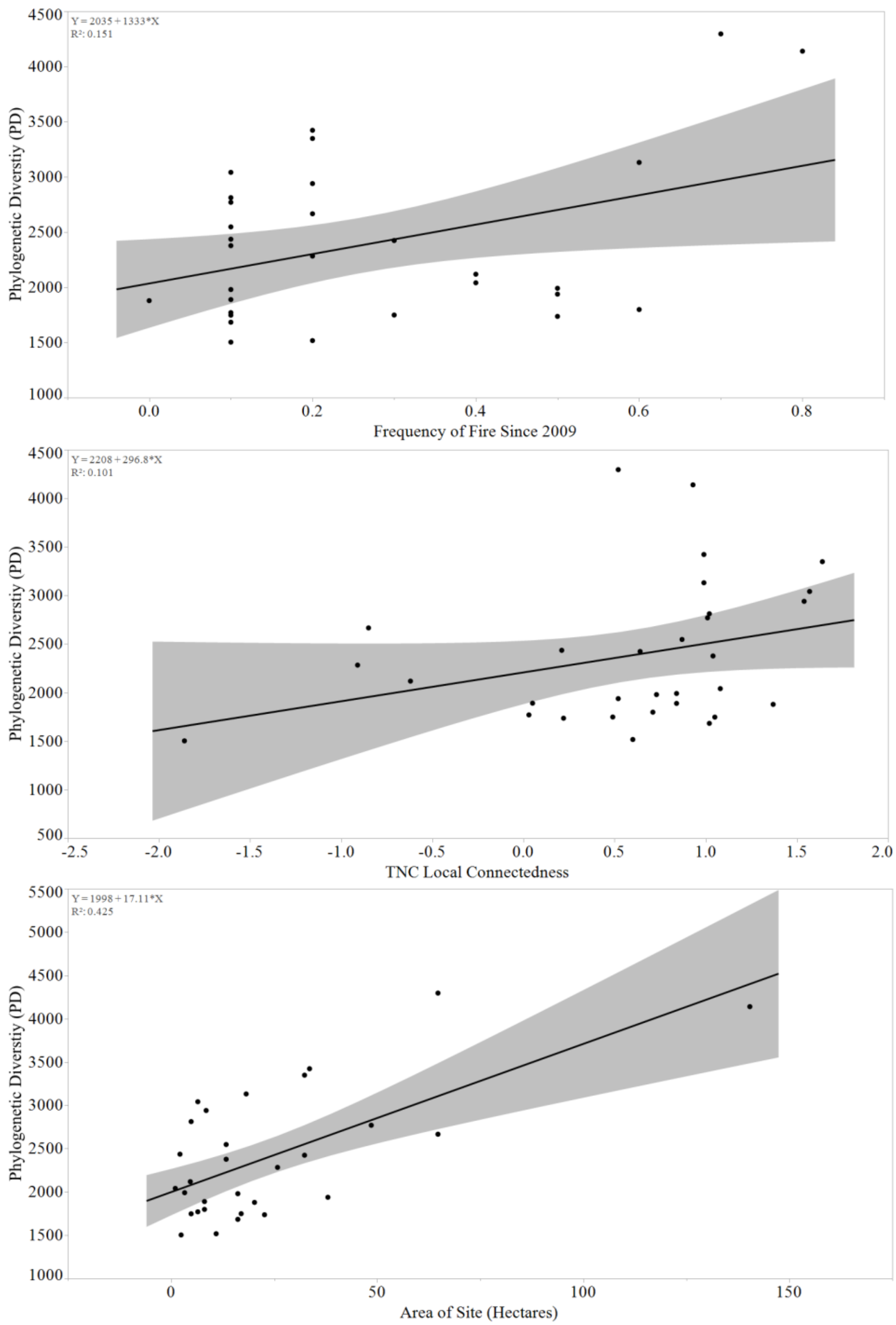

Figure 14. Significant relationships between phylogenetic diversity (PD) and Frequency of Fire (since 2009), TNC Local Connectedness metric and area of the site (in hectares). All three variables indicate a positive correlation with phylogenetic diversity. Shading depicts confidence intervals.

APPENDIX

Code 1. Pruning code used on the Smith & Brown (2018) phylogeny, in order to construct a master prairie survey phylogeny.

```
## Prune tree to southern MN prairie species (built from Phylogenetic Independent Contrasts - Drought Tolerance Common Garden 2013-2014 traits)

## Matt Kaproth 2020-9-17, 2016-09-18 (built off Andrew Hipp's trait/phylogeny script 2014-08-25)


library(ape)

library(adephylo)

library(phylobase)

#library(maticce) ##archeived download

library(phytools)

library(picante)


##### Set working directory setwd("C:/...

#Read in phylogeny, species that overlap

tr <- read.tree('ALLOTB.tre') #Smith and Brown 2019 - AJB - Constructing a broadly inclusive seed plant phylogeny

Spp.names.file <- read.csv("All Surveyed Species (List with Sp ep).csv") #MN survey (excluding 10 unidentified sp. or spp. still in the "All Surveyed Species (List with Sp ep and unknown species).csv that need to be grafted in at end")


# File of corresponding (intersecting) taxa

intersect.taxa <- intersect(tr$tip.label, Spp.names.file$Species) #277 species
```

```
#prune tree to include only intersecting taxa

phylo <- drop.tip(tr, setdiff(tr$tip.label, intersect.taxa)) #creates phylogeny, "phylo" with only

#plot(phylo)

write.tree(phylo, "master_phylogeny.tre") #Write tree being used for PD quantification!


#Identify genera not in intersection

missing.taxa <- setdiff(Spp.names.file$Species, intersect.taxa) #86 species NOT in the tr. Will need to be added in manually.

missing.prairie.genera <- unique(sapply(strsplit(missing.taxa, split = "_"), "[", 1)) #list of 69 genera for species in prairie but not in tr

master.genera <- unique(sapply(strsplit(tr$tip.label, split = "_"), "[", 1)) #list of genera in tr

#missing.master.genera <- setdiff(master.genera, missing.prairie.genera) #large list of genera not in prairie survey

polytomy.master.genera <- intersect(master.genera, missing.prairie.genera) #47 genera present in prairie survey to add to

missing.master.genera <- setdiff(missing.prairie.genera, polytomy.master.genera) #list of 22 genera not in tr
```

Code 2. Master phylogeny created after pruning, and including grafted edits and additions.

(((((((((((Euphorbia_esula:21.907545,Euphorbia_nutans:21.907547):72.299061,Linum_l
ewisii:94.206606):8.401122,((Hypericum_kalmianum:9.086164,Hypericum_ellipticum:9
.086163,Hypericum_perforatum:9.086163):85.779084,(((Viola_sororia:0.006984,Viola_
pedatifida:0.006984):4.586158,Viola_sagittata:4.593142):89.768883,(((Salix_candida:1.
516952,Salix_planifolia:1.516951):5.825448,Salix_interior:7.342401):30.69623,(Populus
_deltoides:4.208234,Populus_alba:4.208235,Populus_tremuloides:4.208235,Populus_spe
cies:4.208235):33.830398):56.323393):0.503224):7.74248):8.819198,(Oxalis_dillenii:0.5
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25.050023)Spermatophyta;

Code 3. Code to calculate Faith's (1992) phylogenetic diversity, based on the master phylogeny.

```
#Prairie Surveys - calculating pd: Faith's Phylogenetic Diversity (a Phylogenetic Community Structure metric)

#MAK and AP 1/27/2020


#Calculate the sum of the total phylogenetic branch length for one or multiple samples. https://rdrr.io/rforge/picante/man/pd.html


#Full detail: pd function calculates Faith's (1992) index of phylogenetic diversity (PD) for each sample in the phylo. Faith's PD index (total branch length among all taxa in a sample, including the root node of the tree) is reported,

#as are the total branch length in the phylogeny, and the proportion of the total branch length in the phylogeny associated with the taxa in each sample.

#References Faith D.P. (1992) Conservation evaluation and phylogenetic diversity. Biological Conservation, 61, 1-10.


rm(list = ls()) #will clear all objects includes hidden objects

library(ape)

library(picante)

library(phytools)

library(gdata) # for Excel

library(pez)   # Will Pearse

library(geiger)

library(nlme)


##### Set working directory setwd("C:/...

##### Load data and clean up a little
```

#species <- read.csv("Kasota Prairie SNA Species List - PD.csv")

tr <- read.tree('master_phylogeny.tre')

#plot(tr);nodelabels(tr$node.label, cex=0.5)

#BigSpecies <- tr$tip.label #make a vector of the big phylogeny species, so a presence matrix file can be made (simlar to phylocom$sample)

#write.csv(BigSpecies, file = "BigSpecies.csv", quote = TRUE,

eol = "\n", na = "NA", dec = ".", row.names = TRUE, fileEncoding = "")

#species <- read.csv("BigSpeciesTable.csv") #You must manually transpose BigSpecies.csv and add in site presence(1)/absence(2) values.

species <- read.csv("Final Big Species Table.csv")

species1 <- sapply(species, as.numeric) #SR will not work if the matrix has factors (characters). Must convert to numeric with sapply.

Calculate Faith's PD #pd(samp, tree, include.root=TRUE)

pd(species1, tr, include.root=TRUE) # Returns a dataframe of the PD and species richness (SR) values for all samples. PD and SR are correlated (only use one of the two)

#Warnings:

#check if tr is rooted - Warning, If the root is to be included in all calculations (include.root=TRUE),

#the PD of all samples will include the branch length connecting taxa in those samples and the root node of the supplied tree.

#The root of the supplied tree may not be spanned by any taxa in the sample. If you want the root of your tree to correspond

#to the most recent ancestor of the taxa actually present in your sample, you should prune the tree before running pd:

prunedTree <- prune.sample(sample,tree)

Set working directory with trial data "phylocom" from pd dataset

```
data(phylocom) #sample file has two columns, one with site, one with species
pd(phylocom$sample, phylocom$phylo) #sample is a numerical matrix
```

Table 1. Management frequency (2009-2019) and invasive species coverage (DF[1, 30]).

Management	Species	F	P
Mechanical	*Phalaris arundinacea*	0.3948	0.5347
Chemical	*Phalaris arundinacea*	0.046	0.8317
Burning	*Phalaris arundinacea*	0.4796	0.4941
Mechanical	*Bromus inermis*	0.314	0.5795
Chemical	*Bromus inermis*	0.5538	0.4628
Burning	*Bromus inermis*	0.102	0.7515
Mechanical	*Rhamnus cathartica*	0.0342	0.8545
Chemical	*Rhamnus cathartica*	0.0059	0.9394
Burning	*Rhamnus cathartica*	0.0005	0.9816
Mechanical	*Melilotus officinalis*	2.5309	0.1225
Chemical	*Melilotus officinalis*	0.0704	0.7927
Burning	*Melilotus officinalis*	1.0872	0.3057

Table 2. Years since last management application (2009-2019) and invasive species coverage.

Management Category	Species	F	P
Mechanical	*Phalaris arundinacea*	0.1995	0.6584
Chemical	*Phalaris arundinacea*	0.367	0.5493
Burning	*Phalaris arundinacea*	0.3467	0.5605
Mechanical	*Bromus inermis*	0.1253	0.7259
Chemical	*Bromus inermis*	0.0308	0.8619
Burning	*Bromus inermis*	0.0003	0.9874
Mechanical	*Rhamnus cathartica*	0.4045	0.5298
Chemical	*Rhamnus cathartica*	0.4828	0.4927
Burning	*Rhamnus cathartica*	0.0005	0.9816
Mechanical	*Melilotus officinalis*	1.1975	0.2828
Chemical	*Melilotus officinalis*	0.1895	0.6665
Burning	*Melilotus officinalis*	0.2984	0.5891
Mechanical	*Melilotus alba*	4.2406	*0.0485**
Chemical	*Melilotus alba*	7.3171	*0.0113**
Burning	*Melilotus alba*	1.5143	0.2284
Mechanical	*Lotus corniculatus*	0.096	0.7589
Chemical	*Lotus corniculatus*	2.6526	0.1142
Burning	*Lotus corniculatus*	2.2179	0.1472
Mechanical	*Cirsium arvense*	0.0002	0.9879
Chemical	*Cirsium arvense*	2.2455	0.1448
Burning	*Cirsium arvense*	2.0057	0.1674
Mechanical	*Berteroa incana*	0.2877	0.5958
Chemical	*Berteroa incana*	0.3261	0.5724
Burning	*Berteroa incana*	0.1626	0.6897