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The Measure of Nematode Diversity in Response to Varying Management Practices and Features in Restored and Remnant Prairie Ecosystems

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THE MEASURE OF NEMATODE DIVERSITY IN RESPONSE TO VARYING MANAGEMENT PRACTICES AND FEATURES IN RESTORED AND REMNANT PRAIRIE ECOSYSTEMS

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A defining moment in American history involved the movement of immigrants across the land looking for space to make a living on and manifest destiny. The Great Plains provided more than enough of this space, and the tallgrass plains making up the eastern part of Nebraska was no exception to this, providing good climate and soils to accommodate bountiful crops. Looking at them now, the loss of tallgrass prairie has been immense. Restoration efforts for the tallgrass prairie have increased, yet it is still a conservation effort needing more analysis and understanding. Evaluation criteria for restored prairies is an important part of this dimension. While above ground assessments make up most of these evaluations, below ground assessments are lacking. To find if below ground diversity measurements can be used as an evaluative measure of prairie restoration success, the nematode family criconematidae was used as an indicator species and compared with four prairies of differing attributes demonstrating restorative quality. Lack of information lowered these qualities to age of prairie, plant diversity, and differences between remnant and restored plots. Using PCR with DNA barcoding, positive correlations were made between the age of prairie and criconematidae nematode diversity, as well as differences found between remnant and restored prairie plots. Comparisons between plant diversity were not significant. While this gives a start to our question, broader research is needed on this topic in order to come to more concrete conclusions to the use of criconematidae nematodes as a bio-indicator of restoration success.

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INTRODUCTION

"After the passage of the Homestead Act in 1862, Early French explorers traversing central North America came upon endless rolling hills of grassland. They had no term for the vast lands they encountered, so they called it prairie; i.e., meadow. The tallgrass prairie region was the first in Nebraska to be settled by Europeans. With its top soil over 18 inches thick and rich in organic matter, as well as the regions ample rainfall, immigrants found this area perfect for growing crops. By 1900, most of the tall grass prairie had been plowed" (Steinauer 2003). Today, "Tallgrass prairie is the most endangered ecosystem in North America" (Rowe et al 2013). To quantify this, "surveys suggest that since European settlement, declines in area of remnant prairie range as high as 99.9%" (Sampson, Fred, and Fritz Knopf 1994). "Remnant prairie is defined as fragments of the original prairie landscape with their native plant communities still intact. Typically, this means soils were never plowed, graded, or buried by fill" (Houseal, Greg). Given this eradication, many of the ecosystem services; i.e., human benefits derived from tall grass prairie, are being diminished as well. These include, but are not limited to: carbon intake, wildlife habitat, flood mitigation, control of agricultural pests, nutrient cycling, watershed protection, and soil preservation (USDA Forest Service). Given these benefits, as well as the extent of eradication which has taken place, restoring and maintaining remnant tall grass prairie should be a conservation priority.

The two general goals involving prairie restoration involves reestablishing the function found in remnant communities such as nutrient cycling, and recreating patterns of plant species abundance and diversity (Polley, H. Wayne, Justin D., and Brian J. Wilsey 2005). With this, an important aspect of restoration is determining how to evaluate the project's effectiveness. Most

prairie assessments involve variable quantitative methods such as the floristic quality index, Shannon's or Simpson's diversity indexes, frequency of woody cover, proximity to other prairie systems, and other above ground assessments. The evaluation of below ground communities or functioning, however, is less considered.

"Soil ecosystems support a diversity of microbes (fungi, bacteria, and algae), micro fauna (protozoa), and mesofauna (arthropods and nematodes)" (Neher 2001). Nematodes, in particular, are the most abundant species on earth and have been used as bio-indicators of soil quality (Neher 2011). They have also been stated as a great indicator tool due to the large amount of information collected on their taxonomy and feeding groups, in comparison to other mesofauna (Neher 2001). The nematode family Criconematidae, has been mentioned in studies as a particularly worthy community to use in studies due to its global distribution, presence in a wide range of habitats, and association with numerous plant species (Powers et al. 2014).

In order to determine whether below ground functioning is associated with restorative processes, or how nematodes can be used as evaluations of restorative success in prairies, this project explores the comparison of criconematid nematode diversity in remnant and restored tallgrass prairies in Nebraska. This research will determine whether this diversity is correlated with specific prairie qualities that give indications of prairie functionality. The qualities being examined are date of initial restoration, the process of restoration (e.g. seed versus seed + sod), distance of the restoration to the nearest native prairie, and current plant diversity. Previous student research projects have addressed the soil diversity within remnant prairies of the region and will serve as a reference for this project. It is hypothesized that higher levels of nematode diversity will be linked with increasing time since the initial restoration, a greater number of plant species, and closer proximity to native prairies.

If nematode diversity relates to the qualities that increase prairie functionality, then nematode diversity within prairies may be able to be used as a biological indicator for the soil community assemblage of prairie restorations. Biological indicators can make it easier for land managers trying to assess the overall quality of their land. Adding nematode diversity as an indicator could increase the available monitoring tools, and may decrease overall costs and energy of management if it allows accurate decision making in the future. In addition to increasing monitoring applications, new species of nematodes are continually being discovered. This study will increase general knowledge of the nematode species in the family *Criconematidae* and how they relate to different prairie ecosystems. As increasingly more land is converted for uses such as agriculture or industry, managers and conservation organizations will be looking to increase restoration of tallgrass prairies. Finding an additional belowground indicator of restored prairie success could indirectly increase prairie restoration achievement.

In conclusion, the purpose of this research is to assess the changes in soil nematode communities following varying factors and levels of restoration in remnant prairie and restored tallgrass prairie. We will determine if older prairie restorations are more diverse. Similarly we will determine if proximity to native remnant prairies influences diversity and in general, if there is a relationship between prairie plant diversity and nematode diversity.

LITERATURE REVIEW

"Nematodes themselves are microscopic worm-like creatures within the soil. A handful of soil will contain thousands of the microscopic worms, many of them parasites of insects, plants or animals. Free-living species are abundant, including nematodes that feed on bacteria, fungi, and other nematodes. In size they range from 0.3mm to over 8 meters" (UNL

Nematology). “While some nematodes can be harmful to plant species such as feeding on plant roots making it more difficult for the uptake of water and nutrients, others are beneficial for plant processes such as nutrient cycling” (Ugarte, Carmen and Ed Zaborski 2014). The family Criconematidae is described as a ring nematode due to their outer body having ring-like, or spiny features (Cordero et al. 2012). “These species are also characterized by having a lip region offset from the body with the presence of one or two lip annuli of different widths, presence or absent of sub-median lobes, annuli margins smooth, crenate or with ornamentation like scales/spines or having an extra cuticle or a sheath covering the whole body” (Cordero et al. 2012).

In terms of using nematodes as an indicator of prairie quality, “Numerous studies showed that soil microorganisms, along with soil free-living nematode communities, have been found to be among the best biological tools for assessing soil disturbances, including heavy-metal pollution , in terrestrial systems” (Pen-Mouratov et al. 2010). In comparison to this, correlations between nematode communities and soil physical and chemical properties have been found. (Kandji, Serlgne et al 2001). Another study looked at nematode community structure in comparison to gradients of desertification in southern, New Mexico. “They found nematodes can be used to identify changes in belowground community structure based on trophic interactions and large-scale disturbances like desertification can have consequences on the diversity and soil biotic functioning at finer spatial scales” (Klass, Jeremy, et al. 2012). Higher levels of below ground diversity have also been found to promote various ecosystem functions, for example, the regulation of microbe communities (bacteria, fungi, protozoa, nematodes, actinomycetes, and algae) and arthropods, recycling of nutrients, and resistance to invasion by exotic species (Bardgett, Richard and Wim van der Putten 2014). This, along with the indicator abilities of

nematodes, suggest a greater possibility of greater nematode diversity correlating with higher prairie fitness.

MATERIALS AND METHODS

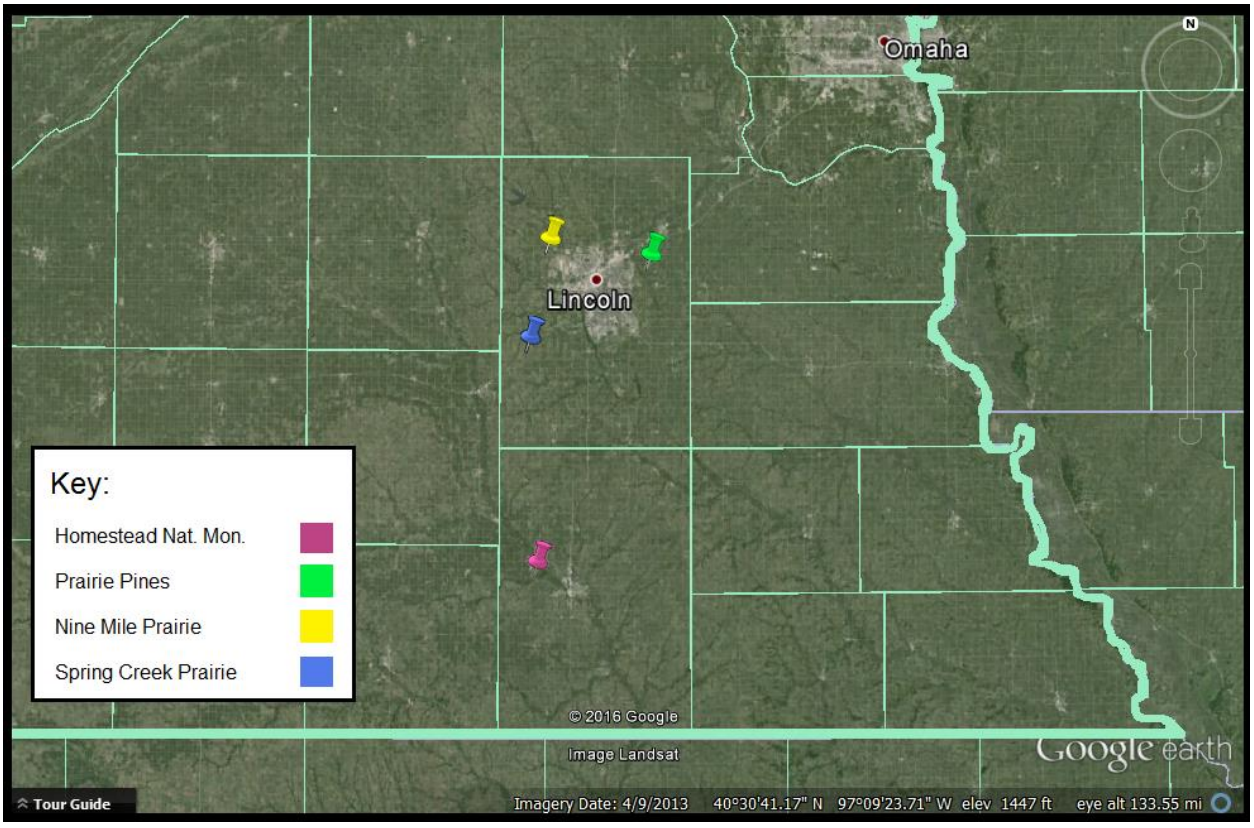
There are a few sampling methods involved with obtaining nematodes. This mainly involves differences in how to obtain the nematodes from the ground, whether this be through the roots of plants or from the soil itself (Zuckerman et al. 1971). The method used in this study involved using the option of soil coring with practices found by other nematology studies to be standardized and sufficient. (Neher et al. 1995). The specific areas being studied are areas of restored and remnant prairies with differing levels of restoration time. Sites chosen include Prairie Pines, Homestead National Monument, Nine Mile Prairie, and Spring Creek Prairie. The map of these sites is found below on Map 1. With the technique used, there are certain steps that should be followed to make sure there is no bias and as less error as possible. "Before samples are collected, a detailed sampling plan, including the collection pattern, and size of cores per sample, and the number of samples are developed" (Zuckerman et al. 1971). "Our field methods includes collecting soil samples extracted by a hand held soil coring tube which are taken systematically within a 40 x 40m grid found using a hand held GPS device. The grids are placed in the average center of the plot being studied. The cores are taken randomly within the grid every 7 paces done by the soil corer. One grid of combined soil cores will constitute an approximately 500cc soil sample, or about 10 soil cores. Once ten cores are collected into a bucket, they are mixed and put into a zip tied plastic bag to keep them preserved. The bucket used is then cleaned so as not to contaminate the next site, and the process is repeated" (Neher et al 1995). GPS, documented history of the plots, and written site notes, are used to identify each

grid for subsequent classification of plant species and prairie quality. Additional information such as management history and restorative factors will be found from representative land managers and literature reviewed on the area being looked at.

After soil samples have been collected, nematodes are separated from the soil using the sieve and centrifugation method (Jenkins 1964). Measures are made on nematode specimens with a Leica DMLB light microscope as well as photo-documentation. Nematode abundances are estimated using a dissecting stereo microscope. This is done by counting the total amount of nematodes in the sample using the microscope and comparing this number with the total amount of criconematidae species in the sample. Five random criconematid species are then chosen to be smashed for DNA analysis. Morphological measurements are taken to be used as indications of in species diversity in addition to DNA evaluations. These include body length, length of esophagus, number of annules and their amounts from excretory pore to the anterior end of the body, and multiple other designated features to try and morphologically ID each nematode species. After these measurements, the nematode is then given an identification number. PCR amplification, DNA sequencing, and DNA Barcode Analysis will be conducted on the select nematode species to identify genotypes that have previously been associated with specific ecological factors (Powers et al. 2014). Within species level of nematode diversity will then be calculated with morphology and haplotype measurements and compared to the different geological and management histories at each prairie. The Simpson's diversity index between three of the prairies will be looked at and compared to haplotype diversity. These values were taken from other comparative studies, cited with figure 4. The Simpson's diversity index quantifies the biological diversity of an ecosystem (Gorelick 2016). For this we are looking specifically at plant diversity. Error bars will be used on graphs making comparisons. "Error bars

demonstrate how confidant you are that these means represents the true value. When error bars overlap this makes the differences between two means not statistically significant, meaning the P-value is greater than 0.05 percent (more than 1 in 20 chance of being wrong)" (Motulsky 1995). Profiles of each sampling site will then be compiled to a region-wide database of prairie soil diversity.

Map 1.



RESULTS

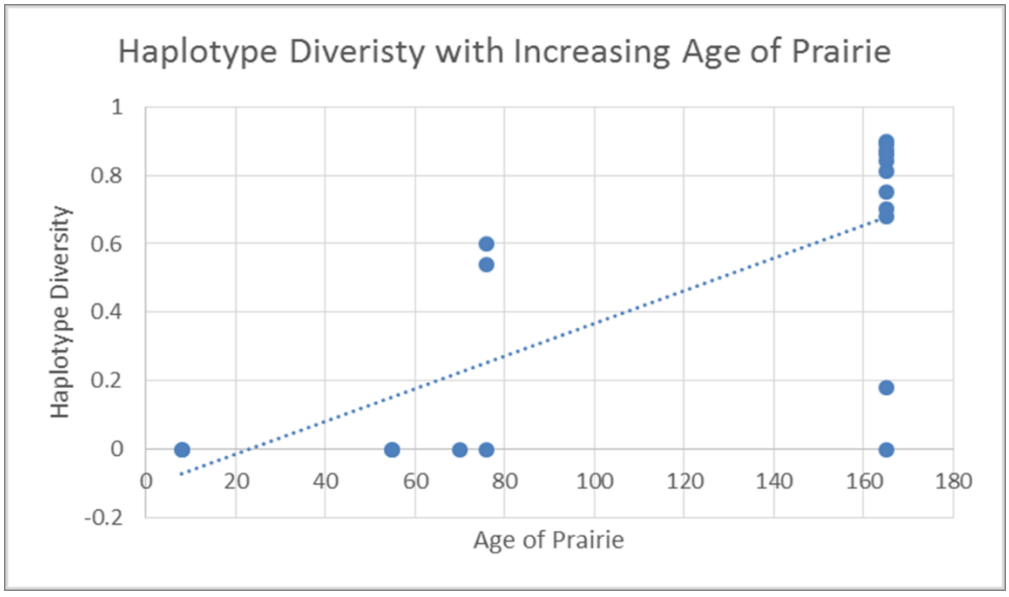
After taking morphological measurements of criconematid species and comparing this with the haplotype measurements found with DNA barcoding, morphology assessments were too vague and undemonstrative to be used as measurements of diversity. With this, haplotype diversity, or diversity using DNA analysis, was the main source of evaluation. Our nearly complete data set can be found in figure 1. This shows the prairies we sampled at or had prior research data from, whether the site was restored or native (remnant), the year management began, years since disturbance (or general age of the prairie), total number of different species, the frequency of different haplotypes, and haplotype diversity measurements. The last column takes the average of haplotype diversity between each prairie site between restored (orange) and native (green) sites in order to make comparisons between haplotype diversity and prairie sites, years since disturbance, and plant diversity measurements. Management between each of these prairies were too close to make any accusations of differences in diversity with varying management practices and a complete map of native prairie sites in my area of study was not available making evaluations of prairie proximity unavailable as well. Due to this, the main factors looked at to compare with haplotype diversity were age of prairie (figure 3), whether the prairie was native or restored, and plant diversity using Simpson's diversity index found from literature review. Each figure is described below for detailed results.

Table 1.

Prairie	Site Description			Total # Different Species	Frequency of Haplotypes (Xi)	Haplotype Diversity H=N(1-ΣXi2)/N-1	Restored(orange) vs. Native (green) (Averaged H)
	Type of Site	Year Type of Management Began	Years since Disturbance	N	ΣXi	H	Haplotype Diveristy Avg,
Homestead National Monument	Restored	1939	76	5	0.52	0.60	0.38
Homestead National Monument	Restored	1939	76	1	1	0.00	
Homestead National Monument	Restored	1939	76	8	0.53	0.54	
Homestead National Monument	Native	1850	165	5	0.44	0.70	0.7
Prairie Pines Preserve	Restored	1960	55	4	0.63	0.50	0.25
Prairie Pines Preserve	Restored	1960	55	4	1	0.00	
Prairie Pines Preserve	Native	1850*	165	1	1	0.00	0.375
Prairie Pines Preserve	Native	1850*	165	8	0.34	0.75	
Spring Creek Audubon Prairie	Restored	2007	8	0	0	0.00	0
Spring Creek Audubon Prairie	Restored	2007	8	0	0	0.00	
Spring Creek Audubon Prairie	Native	1850*	165	7	0.31	0.81	0.495
Spring Creek Audubon Prairie	Native	1850*	165	11	0.83	0.18	
Nine-Mile Prairie	Restored	1945	70	5	0.44	0.70	0.7
Nine-Mile Prairie	Native	1850*	165	7	0.22	0.90	0.84
Nine-Mile Prairie	Native	1850*	165	12	0.37	0.68	
Nine-Mile Prairie	Native	1850*	165	9	0.23	0.86	
Nine-Mile Prairie	Native	1850*	165	8	0.22	0.89	
Nine-Mile Prairie	Native	1850*	165	11	0.21	0.87	
Nine-Mile Prairie	Native	1850*	165	10	0.24	0.84	

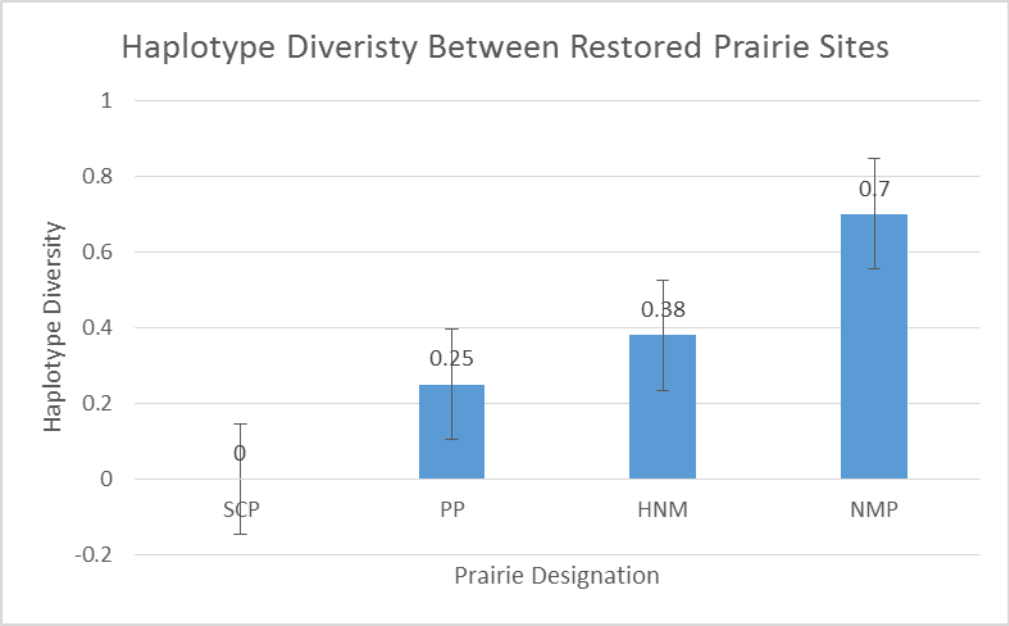
Table 1 is our complete data set of all of our prairie sites, the year they started restoration, the values needed to find the haplotype diversity, and the diversity value itself. The year 1850 was used for native sites since these have technically never been managed, but 1850 being around the time of European settlement.

Figure 1.



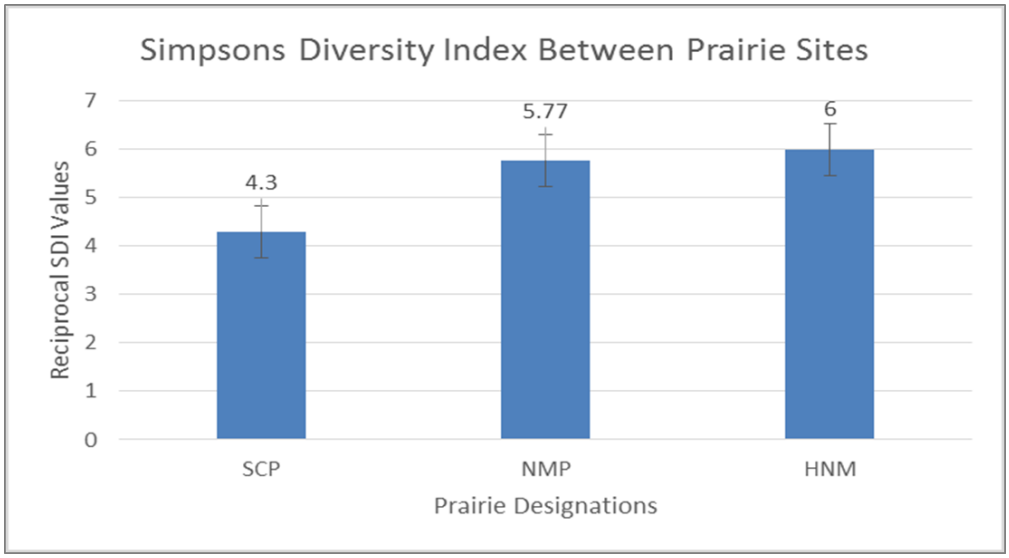
When comparing haplotype diversity with increasing age of prairie, a positively sloping trend line is seen when comparing haplotype diversity with prairie age. Each prairie site also had at least one sample that had no haplotype diversity.

Figure 2.



This graph describes differing haplotype diversity between restored prairie sites. If criconematid diversity reflects restoration success of a prairie, then the restored section of Nine Mile Prairie (the 4th bar line), would be considered the most successful restoration. Looking at the error bars for this graph, we can deduce there is not a statistical difference ($P>0.05$) between Spring Creek Prairie, Prairie Pines, and Homestead National Monument, but there is more statistical difference between that of Nine Mile Prairie and the rest.

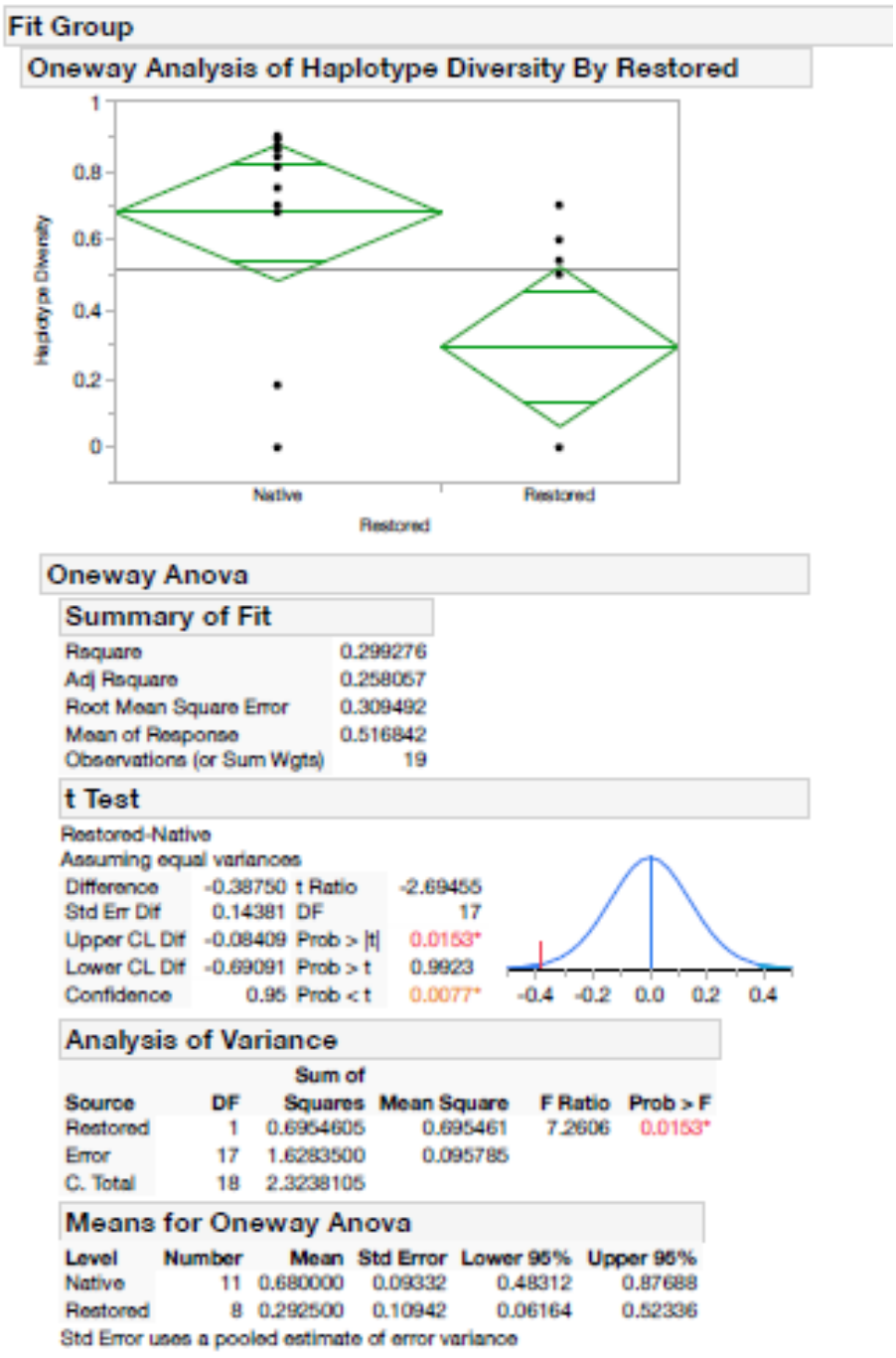
Figure 3.



Data from: (James and Debacker 2007), and (Kottas 2001)

This graph describes the estimated plant diversity using Simpsons Diversity Index (SDI) between three of the prairie sites. These estimations were taken from other sources of literature cited above. Prairie Pines lacks this evaluation due to lack of literature on the topic. There was shown to be lack of statistical evidence ($P>0.05$) between Nine Mile Prairie and Homestead National Monument, but there is more probability of difference between that of Spring Creek prairie and the other two sites. Comparing this with Figure 3 should give some ideas of any correlation between haplotype diversity and prairie plant diversity. Looking at this, it would be assumed Nine Mile prairie would have highest SDI value if haplotype diversity is correlated, yet Nine Mile Prairie and Homestead National Monument both have the highest SDI index scores (Figure 4) instead of Nine Mile Prairie being higher than the rest.

Figure 4.



When comparing total averages for nematode haplotype diversity between restored and native sites, there is statistical evidence of a higher average diversity seen in native sites using a Two-Way-Anova program. The P-Value is less than .05 showing that there is support to show Native sites having higher averaged haplotype diversity than restored ones.

DISCUSSION

This study presents evidence that there is a relationship between nematode diversity and the age of a restored prairie. As shown in Figure 1, there is a positive trend of haplotype diversity of criconematid nematodes and increasing age of the prairie restoration. This study also found, on average, native prairies have more nematode diversity than restored prairies as shown in figure 4. Age of restoration, however, may not be the only factor influencing nematode diversity. Figure 5 suggests loosely that plant diversity may also be correlated with nematode haplotype diversity when looking at how Nine Mile Prairie is one of the two prairies with the highest SDI scores as well as having the highest haplotype diversity average. Having the SDI score of Prairie Pines would make this assessment more valid.

What is clear in this study is that restorations less than 10 years old and prairies converted to agricultural ecosystems have no diversity and generally no criconematid nematodes. While management factors were ultimately too similar, one aspect of management not analyzed in length was that Homestead prairie included sod from native prairie in their restoration. This would assume to increase haplotype diversity within Homestead, but this is only shown by a small degree in comparison with Spring Creek Prairie in Figure 2. This would also likely make haplotype diversity higher than Nine Mile Prairie restorations which did not incorporate sod, but Nine Mile had the highest haplotype diversity between all other prairies looked at.

CONCLUSION

When comparing nematode species diversity with prairie restoration qualities, there are many factors that can influence differing results and conclusions. Considering the time of this project, sampling was done at a very small scale looking at only 4 prairie sites within or close to Lancaster County. The results found are not completely invalid due to this, yet they are only a small aspect of what could be seen with more data collected as well as more literature involved with each site. Considering the small scale of the project, the most demonstrative result found was that of increasing prairie age and haplotype diversity as well as the difference of diversity between native and restored sites. This gives evidence that criconematid diversity can be correlated with how far along the restoration process is in terms of time, as well as higher diversity being found in native sites compared to restored ones. Due to less evidence supporting haplotype diversity and plant diversity, more research should be done to see if above ground diversity can be correlated with the diversity of species below ground. We show in this study that using haplotype diversity of criconematidae species could possibly be a good indicator of restorative success in terms whether or not it is restored or native, as well as an indication of how old a prairie site is, but not as valid in terms of the plant diversity of the site. Overall, more data, as well as more evaluative measures done between these prairie sites would help with future analysis of the use of criconematid diversity as an indication of restoration success.

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