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Wetland Restoration Efforts Result in increasing Phylogenetic Diversity

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ABSTRACT

WETLAND RESTORATION EFFORTS RESULT IN INCREASING PHYLOGENETIC DIVERSITY

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Over the course of ecological restoration efforts, it has been observed that, despite restoration activities, species richness sometimes declines in a given habitat. While this response can be interpreted to mean that restoration activities are ineffective, other measures known as Phylogenetic Diversity Metrics can show that the community is actually becoming more diverse.

Utilizing plant inventories collected as transect data from 1992-2021 of five wetland sites under various types of restoration in northern Illinois, a regional wetland community phylogeny was assembled. The community phylogeny was then analyzed for phylogenetic diversity measures through this 30-year period across the five sites. Additionally, water sampling was performed on the five properties for analyses of water chemistry.

Linearized regression analyses were performed on the phylogenetic diversity metrics. Two of the three metrics showed significant increases in spite of a slight decrease in species richness through time. Additionally, species lists showed a decrease in percent non-native species over time.

One of the active measures of restoration across the five wetland sites was the removal of non-native species, consistent with the finding that the proportion of native to non-native plant species was generally increasing through time. This correlation with increasing phylogenetic diversity metrics suggests that restoration activities have some degree of targeted effectiveness on wetland plant communities.

Supplemental Files:
1. Maximum Likelihood Tree. Created using RAxML-HPC2 on XSEDE v8.2.12.
2. Bootstrap Tree. Created using "Consense" tool from the Phylip v3.66.

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WETLAND RESTORATION EFFORTS RESULT IN INCREASING PHYLOGENETIC DIVERSITY

BY

NICHOLAS T. FOSTER

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INTRODUCTION

Habitat loss and fragmentation has a major impact on different types of ecosystems. In wetlands, habitat loss is often anthropogenic as marshes are drained for agriculture or river straightening is performed to facilitate boat traffic or for misguided attempts to mitigate flooding (Brooker, 1985; McCorvie et al., 1993). Habitat loss compounds other impacts, as less area increases edge effects and reduces resilience of a habitat (Cooper et al., 2012).

Phylogenetic diversity (PD) is increasingly becoming a tool used to evaluate the health of an ecosystem. Phylogenetic diversity (PD) metrics are dependent on how closely related plants species are to one another. PD has been shown to predict susceptibility to invasion and the changes in PD can help inform the efficacy of restoration efforts (Barber et al., 2017; Faith, 1992; Qin et al., 2020). The PD of an ecosystem does not always coincide with simpler measures such as species richness (Barber et al., 2017). Species richness measures the number of species within a community. Phylogenetic diversity should better reflect the health of an ecosystem than less sophisticated metrics.

Wetlands have been impacted in much the same ways as other ecosystem types by habitat loss, pollution, and invasive species. Restoration of wetlands includes some of the same practices as are used for the restoration of other types of ecosystems, but also has unique aspects. Many wetlands in the midwestern U.S. have been converted to agriculture with drainage tiles installed to reduce water levels. Restoration of such wetlands requires that drainage patterns be returned to original conditions. Removal of previously installed drain tiles re-establishes water levels that support the growth of native wetland plants (Hausman et al, 2007).

Pollution has a documented impact on wetland plant community composition. Agriculture pollution and road-salt are consistent inputs into rural and urban wetlands. While research demonstrates the value of wetlands in reducing the concentration of these pollutants in the environment, buffer zones are necessary to decrease the effects of pollution entering into vulnerable habitats (Richburg et al., 2001, Semslitch and Jensen, 2001).

Introduced wetland plants in some cases have substantial impacts on wetland ecosystems and are addressed by specific restoration techniques. For example, *Lythrum salicaria* is a common invader of wetlands (Blossy et al., 2001). As such, the introduction of five different species of insects that consume different parts of the plants has been used as a management technique to attempt to control populations of the invasive plant. Other common invaders of wetlands are *Phalaris arundinacea* (Apfelbaum and Sams 1987), *Typha angustifolia* (Shih et al., 2008), *Iris pseudacorus* (Jacobs et al., 2017), *Phragmites australis* (Rickey et al., 2004), as well as invasive shrubs such as *Rhamnus cathartica* and *Lonicera maacki* (Boyce et al., 2012; Freund et al., 2013). While the reintroduction of a fire regime is effective at controlling the woody invasives, it is not as effective at controlling the invasive forbs and grasses (Grace et al, 2000). Chemical herbicides are commonly used for controlling the wetland invaders.

In this paper three metrics of PD were used to determine if PD is remaining static or changing over time at five wetland sites in northern Illinois. Additionally, changes in percent native species and species richness were calculated for these five wetland sites across the study period. In general, it was observed that PD metrics increased regardless of trends in species richness. My research hypothesis is that phylogenetic diversity metrics will not remain static over the period of restoration in these five wetlands sites in northern Illinois.

METHODS

Five wetland areas were included in this study, having varying degrees of restoration activities applied to them (Table 1). They are all, however, groundwater sourced wetland habitats. Note: water quality measurements were performed on samples from all sites (Tables S1 and S2). The names of these sites, as used in this paper are: Prairie Potholes, Fen Remnant, Fen Sedge Meadow, Ferson Fen, and Parker Fen. For this project, the phylogenetic diversity metrics of the five wetland sites were calculated from transect surveys conducted from 1992 – 2021. Transect surveys were performed during the summer of 2021 along historic transect lines following the methodology of early transect surveys at Parker Fen and the Nachusa Grasslands wetlands.

Three of the transects that contribute to this study are on the property of Nachusa Grasslands, owned by The Nature Conservancy (TNC), a tallgrass prairie nature preserve and restoration in Franklin Grove, Illinois. The Prairie Potholes at Nachusa is converted agriculture land in which, once drainage tile was removed, the water table rose considerably. Now wetland associated species such as *Typha latifolia a*nd *Salix discolor* dominate this site as well as well as less frequent occurrences of *Rumex orbiculatus, Caltha palustris,* and *Eutrochium maculatum*.

The Fen Remnant and Fen Sedge Meadow, both at Nachusa, are adjacent to one another, separated by a strip of *Quercus* dominated woodland. The Fen at Nachusa is adjacent to a spring fed stream. The bank of the stream is heavily incised, and is infiltrated with tunnels created by beavers. This area is dominated by *Carex* sp. and *Pycnanthemum virginianum.* The flora of the Fen Sedge meadow is notably similar to that of the Fen Remnant. The Nachusa transect data was provided by Dr. E. Bach (The Nature Conservancy, Illinois Chapter). A permit to conduct research at The Nature Conservancy's Nachusa Grasslands in 2021 was obtained.

All three Nachusa transects are subject to similar restoration practices, i.e., removal of invasive plants through chemical control and targeted removal of woody plants. Reintroduced bison also have had free rein of the property since 2016.

Parker Fen is a privately owned spring fed wetland near Woodstock, Illinois surrounded by woodland. This fen is populated with *Eleocharis rostellata*, *Lycopus uniflorus,* and *Typha* sp. Woodland encroachment and *Typha* colonization are plaguing this secluded wetland. The Illinois Nature Preserves Commision (INPC) provided the transect survey data for Parker Fen (INPC director, John C. Nelson). Permission of the landowner, Ms. J. Haun, and an INPC Special Use permit was approved 19 July 2021 prior to the 2021 survey of the privately-owned Parker Fen.

Ferson Creek Fen is located between the Fox River and Illinois Route 31 near St. Charles, Illinois and owned by the St. Charles Park Distirct and managed by the Naturalist Department. Impacted by flooding and pulse hydrology from the river, restoration efforts are focused on reducing the populations of *Lythrum salicaria*, *Typha* x *glauca*, and woody plants. Historic transect data (1992-2019) for Ferson Creek Fen were acquired directly from the landowner. A permit was not required for Ferson Creek Fen as the last previous transect survey, conducted by B. Johnson (botanist contracted by the St. Charles Park District) in 2019, was used for this study.

Table 1. Summary of restoration activities at five wetland study sites in northern Illinois.

Site	Prescribed Burns	Herbicide Targets	Planting	Additional Restoration Measures
Ferson Creek Fen	Sporadic	Shrubs, Forbs, and grasses	Plugs and Seed sourced from site	Loosestrife Beetle Introduced
Parker Fen	None	Shrubs	None	None
Potholes	Consistent	Grasses	Seeding	Drain Tile Removed, Bison introduced 2014
Fen Sedge Meadow	Consistent	Grasses, shrubs	Seeding	Bison Introduced 2014
Remnant Fen	Consistent	Grasses, shrubs	Seeding	Bison Introduced 2014

At all five sites, quadrats were spaced along transects and all plant occurrences were recorded. Although there was some variability in spacing and size of quadrats, at each site a method consistent to what was done in the past was used to conduct transect surveys (summarized in Table 2). Plant occurrence and cover values were recorded on each transect except for the 2021 Parker Fen Transect, however, the method of cover value reporting was not consistent between sites and not used in this study. Surveys from all five sites and a species inventory for Parker fen were combined to produce a data matrix of 269 plant species for the summary wetland community phylogeny. For these species sequences of two widely-used gene sequences, *matK* and *rbcL*, were downloaded from the Genbank database (https://www.ncbi.nlm.nih.gov/) when available, including those from complete plastomes. These two genes were selected for their availability of sequences, being two of the most sequenced plastomes available on NCBI, and are effective as barcode sequences in calculating

PD (CBOL Plant Working Group, 2009). Of the 269 original species there were 259 *matK* and 265 *rbcL* sequences.

Table 2. Details of transect surveys consistently applied for each of six wetland sites.

Site	Number of Quadrats	Quadrat Size	Quadrat Spacing
Ferson Creek Fen	20 Quadrats	1/4 m^2	10 paces
Park Fen	20 Quadrats	1/4 m^2	5 meters
Nachusa Fen Remnant	15 Quadrats	1 m^2	5 meters
Nachusa Fen Sedge	15 Quadrats	1 m^2	5 meters
Nachusa Prairie Pothole	15 Quadrats	1 m^2	5 meters

The *matK* sequences and the *rbcL* sequences were aligned separately using MAFFT v7.450 (Katoh & Standley, 2013). Then the two alignments were concatenated in Geneious v11.1.5 (Biomatters; Kearse et al., 2012). The concatenated alignment was then uploaded to the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010) and a maximum-likelihood (ML) analysis was performed using RAxML-HPC2 on XSEDE v8.2.12 (Supplemental File 1; Stamatakis, 2014), with *Lycopodioides apoda* selected as the outgroup. A ML bootstrap analysis was performed with 1000 replicates. The consensus bootstrap tree was constructed using the "Consense" tool from the Phylip v3.66 software suite (Supplemental File 2; Felsenstein, 2005)— that was accessed from the CIPRES science gateway (Fig S2 and S3).

The picante package (Kembel et al., 2010) for the R software suite, v. 3.6.2 was used to estimate three metrics of phylogenetic diversity for each transect, standardized effect size of phylogenetic diversity (PD), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD). These metrics determine the phylogenetic evenness or clustering of the species that comprise the transect survey at each year. To control for variation in species richness, standardized effect size compares the observed ratios of randomly generated sets of species. These randomizations were performed 10,000 times and averaged.

Linearized model analysis was performed in R using ggpubr and plotted suing ggplot2 (Wickman, 2016; Kassambara and Kassambara, 2020) using the year the transect data were collected as the independent variable and each of the diversity metrics as dependent variables (Fig. 1). Additionally, a linearized model analysis was performed with percent native species that appeared on each transect.

RESULTS

Quadrats surveyed in 2021 matched the number of quadrats in the earlier transects. Twenty quadrats were surveyed at Parker Fen, resulting in a species richness of 43, greater than that of the 1999 survey, which was 34. The Nachusa sites, Prairie Potholes, Fen remnant, and Fen Sedge each had 15 quadrats surveyed, with species richnesses of 24, 45, and 38 respectively. Earliest available transect data for the Nachusa sites had species richnesses of 24, 34, and 28 respectively. A 2016 survey of the Fen Sedge site had a species richness of 43 and a 2015 survey of the Prairie Potholes had a species richness of 24. Surveys conducted at Ferson Fen in 1992, 1997, 2002, 2007, and 2019 had richnesses ranging from 47 to 85.

The ML phylogenetic tree is shown in Fig. S1. The Bootstrap majority-rule consensus tree is shown in Fig. S2. Major clades corresponded to widely recognized taxa (Judd et al., 2016). Monocots were supported with a bootstrap value of 84% with the Poales clade having 99% BV, and Poaceae and Cyperaceae both had 100% bootstrap values. Two major clades within the Eudicots were monophyletic, with Rosids having a 66% bootstrap value and Asterids having a 75% bootstrap value. Within Asterids, Asterales had a BV of 96%. Within Rosids, Rosales had a BV of 86%. Notably, *Cuscuta gronovii*, while appearing in an expected clade, has the longest terminal branch in the phylogenetic analysis. This is likely due to it being a parasitic plant with a highly modified plastid genome, including a truncated copy of *mat*K (Funk et al. 2007). Some relationships in the community phylogeny were unexpected, especially for deep nodes in the tree where phylogenetic information from the two loci was limiting. For example, a clade of six species of Ranunculales was sister to the large clade of monocots rather than being united with eudicots, but support for this relationship was low (BV 54%). Phylogenetic positions among nodes toward the termini of the tree were only occasionally unexpected, such as the position of *Symphyotrichum novi-belgii*, which was embedded in a clade of nine *Solidago* spp. (BV 64%).

Results from the linearized regressions are shown in Fig. 1 and Table 3. MNTD and PD show an increase over the sampling period with *R* values of 0.46 and 0.74, respectively and *p* values of 0.084 and 0.0018, respectively. MPD also showed an increase over the sampling period and species richness declined over the same period, but neither of these trends were statistically significant.

Table 3. Phylogenetic diversity metric results for five community assemblages based on the overall wetland community phylogeny of Figure 1.

Year and site	ntaxa	mpd.obs	mpd.obs.z	mpd.obs.p
1992 Ferson Creek Fen	61	16678.85	-1.9097936	0.03419658
1993 Ferson Creek Fen	49	15955.25	-2.6333382	0.0059994
1994 Ferson Creek Fen	42	15031.68	-3.5605869	0.00049995
1995 Ferson Creak Fen	57	14971.84	-4.3529008	0.00019998
1996 Praire Potholes	23	18091.3	0.143191	0.5370463
1996 Ferson Creak Fen	54	16887.12	-1.5023197	0.069993
1997 Ferson Creek Fen	66	16576.85	-2.2079877	0.01769823
1997 Nachsua Fen Remnant	34	17170.29	-0.8370893	0.19608039
1997 Nachusa Fen Sedge	27	19638.66	1.59876	0.95040496
1998 Ferson Creek Fen	81	16312.44	-3.0196824	0.00159984
1999 Parker Fen	30	17826.9	-0.1078806	0.43685631
1999 Ferson Creek Fem	86	17996.2	0.1399962	0.54914509
2002 Ferson Creek Fen	77	16731.33	-2.1442302	0.01689831
2007 Ferson Creek Fen	75	16769.42	-2.031859	0.02609739
2015 Nachusa Prairie Pothole	23	119625	0.6932212	0.7490251
2016 Nachusa Fen Sedge	40	148130	-2.0891342	0.01989801
2019 Ferson Creek Fen	44	16558.53	-1.7420092	0.04839516
2021 Nachusa Fen Remnant	37	17419.34	-0.5947401	0.27287271
2021 Nahcusa Prairie Potholes	23	16185.3	-1.485823	0.07389261
2021 Parker Fen	38	18242.72	0.3517909	0.62783722
2021Nachusa Fen Sedge	33	17795.42	-0.1458336	0.43545645

Continued on following page

Table 3, continued.

Year and site	ntaxa	mntd.obs	mntd.obs.z	mntd.obs.p
1992 Ferson Creek Fen	61	4101.443	-0.9992601	0.15893411
1993 Ferson Creek Fen	49	4384.531	-1.1741044	0.12088791
1994 Ferson Creek Fen	42	4980.024	-0.1099467	0.45580442
1995 Ferson Creak Fen	57	3891.263	-2.3074689	0.009999
1996 Praire Potholes	23	5371.783	-1.56249314	0.05769423
1996 Ferson Creak Fen	54	4396.315	-0.5581356	0.28637136
1997 Ferson Creek Fen	66	3931.394	-1.28047352	0.10078992
1997 Nachsua Fen Remnant	34	5559.471	0.28547344	0.61188881
1997 Nachusa Fen Sedge	27	4947.185	-1.94756988	0.02609739
1998 Ferson Creek Fen	81	3806.321	-0.3043242	0.37556244
1999 Parker Fen	30	6218.233	1.06221221	0.85361464
1999 Ferson Creek Fem	86	3552.884	-1.3824919	0.08269173
2002 Ferson Creek Fen	77	3650.545	-1.6838993	0.04579542
2007 Ferson Creek Fen	75	3925.56	-0.3220933	0.37536246
2015 Nachusa Prairie Pothole	23	7379.174	1.7442495	0.9544046
2016 Nachusa Fen Sedge	40	4855.175	-0.7303536	0.2393761
2019 Ferson Creek Fen	44	5047.932	0.38212355	0.64643536
2021 Nachusa Fen Remnant	37	5006.784	-0.69708729	0.24852515
2021 Nahcusa Prairie Potholes	23	6368.043	0.05753424	0.52524748
2021 Parker Fen	38	5535.395	0.84759144	0.79922008
2021Nachusa Fen Sedge	33	5408.818	-0.23853093	0.40775922

Continued on following page.

Table 3, continued.

Year and site	ntaxa	pd.obs	pd.obs.z	pd.obs.p
1992 Ferson Creek Fen	61	200786	-1.28768739	0.09949005
1993 Ferson Creek Fen	49	176322	-1.03353085	0.15123488
1994 Ferson Creek Fen	42	166569	0.01101941	0.50074993
1995 Ferson Creak Fen	57	189409	-1.75977359	0.04049595
1996 Praire Potholes	23	102012	-1.98949003	0.02509749
1996 Ferson Creak Fen	54	190278	-0.5820112	0.27932207
1997 Ferson Creek Fen	66	207104	-1.94991665	0.03059694
1997 Nachsua Fen Remnant	34	144453	-0.29950285	0.37761224
1997 Nachusa Fen Sedge	27	119281	-1.181614	0.11808819
1998 Ferson Creek Fen	81	246534	-0.1761646	0.42760724
1999 Parker Fen	30	134485	-0.16523979	0.43235676
1999 Ferson Creek Fem	86	252895	-0.63211287	0.25767423
2002 Ferson Creek Fen	77	230990	-1.54981953	0.06209379
2007 Ferson Creek Fen	75	235612	-0.11344772	0.44835516
2015 Nachusa Prairie Pothole	23	17188.4	-0.6355261	0.25577442
2016 Nachusa Fen Sedge	40	16818.23	-1.3449333	0.09359064
2019 Ferson Creek Fen	44	175647	0.682485	0.75042496
2021 Nachusa Fen Remnant	37	156469	0.36786343	0.63818618
2021 Nahcusa Prairie Potholes	23	114052	-0.16285746	0.43165683
2021 Parker Fen	38	158287	0.26264302	0.59619038
2021Nachusa Fen Sedge	33	143223	-0.08545296	0.46120388

A linearized regression of the percentage of native plants to non-native plants showed an increase in percent native plants across the sampling period, which coincided with increased PD metrics, with an *R* value of 0.5 and a *p* value of 0.056.

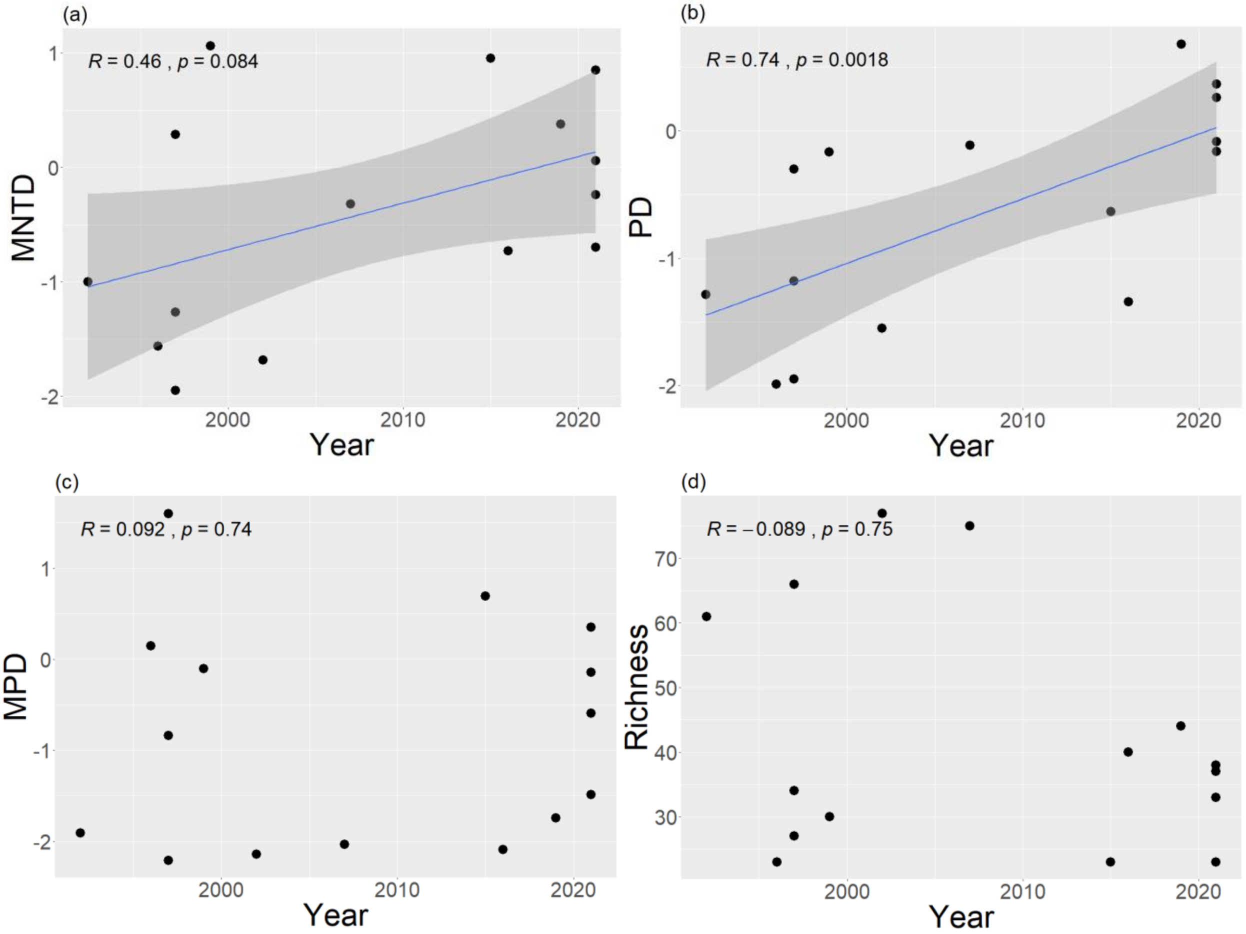

Fig. 1: Linearized regressions show changes in diversity metrics over time across five sites. *R* and *p* values are shown on each plot. Mean nearest taxon distance (a) and phylogenetic diversity (b) increased. Mean pairwise distance (c) and species richness (d) showed no significant change.

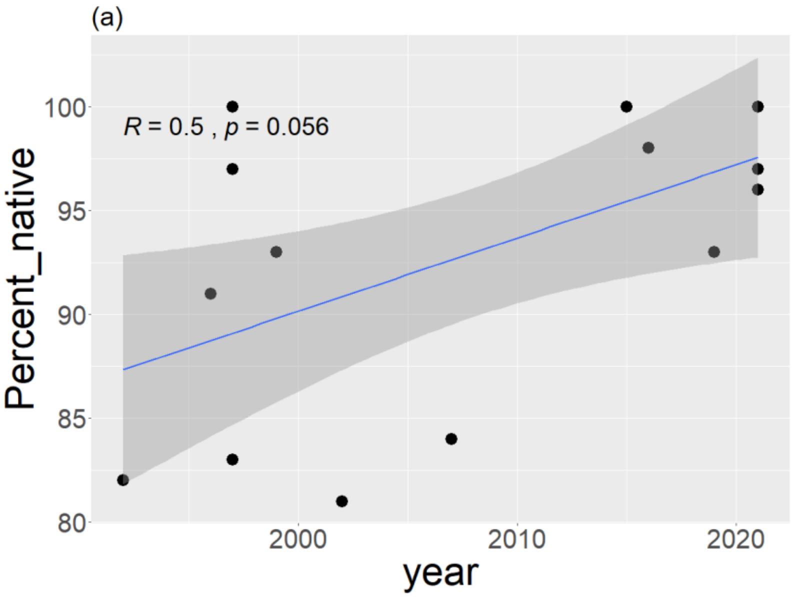

Fig. 2: Linearized regressions show the change in percent native plant species over time across five sites. *R* and *p* values are shown on the plot. Note that percent native plant species increased significantly over the sampled time period.

DISCUSSION

Two of the three PD metrics increased in my analyses supporting my hypothesis that restoration efforts correlate with observable changes in values of PD at the five wetland sites in my study. Species richness did not show a correlated increase across the study period. Concurrently, the proportion of native to non-native species also increased across sites during the study period.

Phylogenetic Diversity has been previously utilized to demonstrate the efficacy of restoration activities and can show trends in community structure that are otherwise undetected by species richness alone (Barber et al., 2017). Phylogenetic Diversity metrics have only recently been applied to wetlands. Wetlands provide critical habitat for numerous organisms including endangered plants and animals (Kiviat, 1997). Wetlands are also exceptional at absorbing pollutants, particularly agricultural run-off, and are also effective in reducing flooding impacts by absorbing the flood waters (Sutton-Grier and Sandifer, 2019). The importance of wetlands is recognized as the USDA announced it would be investing $5 million in wetland mitigation banking, grants that could be utilized in establishing new wetlands or for restoration efforts in existing ones (USDA News Release, 2021).

Some previous studies used published evolutionary trees to expedite the PD analysis (Lishawa 2019; Zhukov 2017). In this study a community phylogeny data matrix was newly assembled for 269 plant species obtained in transect surveys conducted over a 30-year period. Using a tree that was inferred from the community of plants occurring in the study sites more accurately focused the analytical process on those species in the regional wetland ecosystems.

The regression analyses of PD and MNTD showed an increase over time while MPD and species richness showed no significant change (Fig 1). This suggests that restoration efforts are having a generally positive impact on PD possibly not reflected in the simpler measure of species richness.

While species richness showed no significant change over time, the ratio of native species to non-native species increased, which correlates with the finding of increasing PD metrics. Nonnative invasive species, particularly in wetlands, outcompete native flora, either through generating massive amounts of seed or by colonizing large swaths of land through rhizomes. With restoration activities focusing on nonnative species removal, this finding suggests those efforts are effective. Increasing MNTD and PD may be representing decreases in non-native and abundant species and incursions by phylogenetically diverse native species. Incorporating abundance data and cover values could help determine if the increases in PD metrics is a result of the restoration activates of removing key invasive plant species.

While the timeline of the surveyed transects spanned nearly 30 years, their frequency dropped off in the 2000s. It is unknown how the significance of PD metrics might change given more consistency in the transects. Future studies could include more wetland sites with regular transect surveys and incorporate abundance data in analysis to refine our understanding of how changes in PD metrics reflect responses to restoration measures.

Restoration and conservation of natural habitats is essential to combating the ongoing biodiversity crisis. Habitat fragmentation, invasive species, pollution, and climate change are all anthropogenic impacts that, through restoration efforts, can be curbed. Understanding how communities are changing in response to anthropogenic inputs is vital to future restoration efforts and this study shows that phylogenetic diversity is an effective metric to determine changes in plant diversity that species richness, an easier tool for land managers to implement, could be interpreted as a failure in restoration efforts.

CONCLUSIONS

Utilizing transect data collected over the span of almost 30 years, I was able to demonstrate that restoration efforts have had a measurable impact on the phylogenetic diversity of the wetland plant communities, which is different than the response of species richness. With most of the restoration efforts focusing on non-native species removal, I found the proportion of non-native species was declining. This correlation with increasing phylogenetic diversity metrics suggests that restoration activities are having positive impacts on wetland plant communities.

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APPENDIX

WATER QUALITY SAMPLING AND MEASUREMENTS

WATER QUALITY SAMPLING AND MEASUREMENTS

Methods

Between August and October of 2021, the five wetland sites were sampled using a Hach 40Qd probe on site, where pH, Oxidation Reduction potential, dissolved oxygen, and total dissolved minerals were recorded (Table S1) according to EPA Method for each probe (Hach, 2021). Then, samples of the water were collected that were then tested for the chemicals listed in Table 2. The levels of the chemicals in ppm were determined using a handheld eXactMicro photometer and corresponding test strips, with the procedure for each test outlined in its user manual. Parker fen was sampled on three occasions, while the other sites were sampled just twice.

The three Nachusa sample sites are a seep, where ground water that feeds into both the Fen sedge meadow and Fen remnant transects is the highest, the creek adjacent to the fen sedge transect, and pothole, water in the pothole transect at Nachusa.

Table S1: Results of pH, electric conductivity (EC; µS/cm), dissolved oxygen (DO; mg/l) and oxidation reduction potential (ORP; mV)

Site	**Date**	**pH**	**EC µS/cm**	**DO mg/l**	**ORP mV**
Seep- Nachusa	8/2/2021	6.76	607	7.27	151.8
Creek-Nachusa	8/2/2021	7.16	515	7.54	143.6
Pothole-Nachusa	8/2/2021	6.75	498	7.34	35.3
Ferson	8/25/2021	7.1	904	6.22	-315.4
Parker Fen	8/27/2021	7.05	1072	7.58	-1725
Parker Fen	9/17/2021	6.88	1137	6.83	14.2
Ferson	10/10/2021	6.63	1084	2.64	-292.3
Parker Fen	15/10/2021	6.76	1024	8.25	-215.4

Table S2a: Results of eXact® Micro photometer in ppm. S^- is sulfide.

Site	**Date**	**NH_4^+**	**NO_2^-**	**NO_3^-**	**SO_4^{2-}**	**S^-**	**PO_4^{3-}**
Seep- Nachusa	8/2/2021	0.89	0.54	15.8	23	0.19	1.65
Creek-Nachusa	8/2/2021	0.18	0.09	10.4	0	0.05	0
Pothole-Nachusa	8/2/2021	0.98	0.28	10.6	36	0.11	2.11
Ferson	8/25/2021	1.76	>1.8	>30	0	0.05	0
Parker Fen	8/27/2021	0	4.43	0	0	0	lo
Parker Fen	9/17/2021	0.19	Hi	lo	25	0	lo
Pothole Nahusa	9/24/2021	0.46	0	11.9	18	16	1.62
Seep- Nachusa	9/24/2021	0.4	0.18	44	21	0	0.39
Creek-Nachusa	9/24/2021	0.16	0.07	64	55	0.04	0.38
Ferson	10/10/2021	0.15	0.03	9.7	12	0.07	0.47
Parker Fen	15/10/2021	0	0	lo	67	0	0.31

Table S2b: Results of eXact® Micro photometer in ppm. THH = Total Hardness, High; Fe = Iron, total; Br = Bromine (total); CH = Chloride (as NaCl). A result of "lo" means the concentration was below a detectable level.

Site	**Date**	**Ca^{2+}**	**THH**	**Fe**	**AL**	**Al^{3+}**	**CH**	**Br**	**Mn^{2+}**	**Cu^{2+}**
Seep-Nachusa	8/2/2021	222	416	2.8	0.15	-	109	1.78	-	2.65
Creek-Nachusa	8/2/2021	138	254	2.21	0	-	64	0	-	0
Pothole-Nachusa	8/2/2021	221	543	3.6	0.41	-	152	4.5	-	3.41
Ferson	8/25/2021	>400	>600	2.12	0.41	0.12	85	0	0	0
Parker Fen	8/27/2021	>400	lo	2.27	376	0.04	256	0.04	0	>10
Parker Fen	15/10/2021	-	910	-	-	-	-	-	-	-

Discussion

Results from the eXact® Micro photometer that contain a ">" means concentration of those pollutants were higher than the device was able to read. To determine the actual concentration a dilution would have had to been performed. During the early stages of collection, this step was neglected, and results were recorded as being greater than the maximum concentration readable by the device. Given the low sampling frequency, these measurements are included in my results. A "lo" result is effectively 0, being to low a concentration to detect.

Future research could utilize multivariable linear analysis to determine if water quality has an impact on wetland plant PD. It has been observed that prescribed burning can have a measurable impact on nutrients in water, which could account for the higher level of NH4 at Ferson Creek Fen and the three Nachusa samplings compared to Parker Fen (Battle and Golladay, 2003). High electric conductivity at Parker Fen and Ferson Creek Fen could be a result of salt inputs into the environment. Parker Fen having past issues with water softener discharging into the fen and Ferson's proximity to the Fox River and a busy road, road salt could be inputs that raise the electric conductivity. A 1997 report on groundwater contamination into Illinois nature preserves reported Ferson Creek Fen had a pH of 9.0 and an oxidation reduction potential (ORP) of 580 and an electric conductivity of 760. Parker Fen, in that same report, had a pH of 7.6, an eclectic conductivity of 720, and an ORP of –28 (Locke and Mushrush, 1997). These results are drastically different, especially pH for Ferson, and both ORP and electrical conductivity for both sites. The Nachusa sites were not included in this report as Nachusa had not yet been granted nature preserve status.

Water chemistry and hydrology are important factors to consider in the ecosystem functioning of wetlands. Microorganisms, particularly mycorrhizal fungi, nitrogen-fixing bacteria, and those species causing decomposition and turnover of nutrients, are most directly impacted by factors such as pH, EC, ORP, and other aspects of hydrology. In turn, wetland plants that depend on the activities of these microbes could be affected, with impacts on their persistence and prevalence in the ecosystem. However, given the low sample size, my confidence that these results reflect the true quality of the water is not high. If the changes in pH, EC, and ORP at Ferson and Parker fens are evidence of declining water quality, this decline does not appear to be reflected in Phylogenetic diversity changes.

Utilizing the water quality collected in 2021 could be used to evaluate a correlation between the phylogenetic diversity and water quality, particularly electric conductivity, oxidation/reduction potential, and nutrients (including nitrates and nitrites) in the system. Ideally, more data points for these parameters would be collected to strengthen the confidence in the true quality of the water in these systems.

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Table S3. Accession numbers for *mat*K and *rbc*L sequences obtained from GenBank for 267 species. When *mat*K and *rbc*L accession numbers are the same for a given taxon, full-length sequences for each locus were extracted from complete, banked plastomes rather than from individual locus sequences. Synonyms for taxon names, sometimes used in GenBank, appear parenthetically following the accepted names.

Species	***mat*K**	***rbc*L**
Acer negundo	MN864501.1	MN864501.1
Acer saccharinum	MW067071.1	MW067071.1
Acorus americanus (*A. calamus*)	EU273602.1	EU273602.1
Agalinis purpurea	JX091307.1	EU828238.1
Agalinis tenuifolia	AY563916.1	AY563936.1
Agrimonia gryposepala	HQ593157.1	HQ589939.1
Alisma triviale	MG216964.1	HQ901563.1
Alliaria petiolata	NC_049586.1	NC_049586.1
Allium cernuum	MT348440.1	MT348440.1
Ambrosia artemisiifolia	MG019037.1	MG019037.1
Ambrosia trifida	MG029118.2	MG029118.2
Amphicarpaea bracteata	EU717399.1	None
Andropogon gerardii	MH181178.1	MH181178.1
Anemone canadensis	KM364772.1	MN601432.1
Angelica atropurpurea	None	KF613025.1
Apios americana	KF856618.1	KF856618.1
Apocynum sibiricum	MK509432.1	MN601434.1
Aquilegia canadensis	EU827653.1	AY392755.1
Arisaema dracontium	KC466573.1	KC466585.1
Arisaema triphyllum	KC466577.1	AY298817.1
Asarum canadense	MG544848.1	MG544848.1
Asclepias incarnata subsp. *pulchra*	MG678864.1	MG678864.1
Bidens cernua	JN893870.1	HQ589973.1
Bidens frondosa	NC_050965.1	NC_050965.1
Bidens trichosperma (*Bidens coronata*)	AY551476.1	MG222034.1
Boehmeria cylindrica	MG220667.1	KJ773314.1
Bolboschoenus fluviatilis (*Scirpus fluviatilis*)	MG217063.1	GQ130358.1
Bromus ciliatus	MF597203.1	JX848490.1
Bromus pubescens	MF597238.1	MG225580.1
Calamagrostis canadensis	DQ786896.1	MF596671.1
Caltha palustris	NC_041532.1	NC_041532.1
Calystegia sepium (*Convolvulus sepium*)	FJ395438.1	AY100992.1

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Table S3, continued		
Campanula aparinoides	LT706755.1	EU643728.1
Cardamine bulbosa	MK519738.1	MG249170.1
Carex aquatilis	KP273666.1	MG226954.1
Carex blanda	KJ513583.1	HQ589988.1
Carex buxbaumii	LK021891.1	MG228098.1
Carex comosa	HG915847.1	MG227826.1
Carex grisea	FJ597215.1	MG227492.1
Carex haydenii	MK519799.1	MG226929.1
Carex hystericina	HG915855.1	HQ589998.1
Carex interior	KX677231.1	HQ589999.1
Carex lacustris	MK519806.1	MG227665.1
Carex lasiocarpa	HG915858.1	JX644625.1
Carex leptalea	KR902913.1	KJ773353.1
Carex pellita	MK519832.1	MG225706.1
Carex prairea	MK519836.1	MG226818.1
Carex projecta	MK519838.1	KF977472.1
Carex retrorsa	HQ593216.1	HQ590010.1
Carex sartwellii	MK519844.1	MG228242.1
Carex scoparia	MN762964.1	MG191387.1
Carex sterilis	MK519851.1	MG227857.1
Carex stipata	KJ513593.1	KJ773358.1
Carex stricta	MK519854.1	MG227578.1
Carex suberecta	MK519855.1	MG226508.1
Carex tetanica (*Carex meadii*)	MK519859.1	MG227165.1
Carex trichocarpa	HG915882.1	MG227271.1
Carex vulpinoidea	KP273710.1	HQ590018.1
Celastrus orbiculatus	EU328945.1	AJ235775.1
Chelone glabra	HQ593231.1	HQ384895.1
Cicuta bulbifera	MG224973.1	MG223080.1
Circaea canadensis (*Circaea lutetiana* var. *canadensis*)	KJ592900.1	MF349680.1
Cirsium arvense	KC969499.1	JX848406.1
Cirsium discolor	MG225067.1	MG224601.1
Cirsium muticum	MK519921.1	MG222572.1
Cladium mariscoides	MK519925.1	MG225556.1
Clematis virginiana	MG221097.1	HQ590040.1
Cornus obliqua	U96898.1	MG222178.1
Cornus racemosa	DQ340470.1	DQ340445.1
Cornus sericea (cornus stolonifera)	EU749305.1	AY725857.1
(Continued on following page)		

Table S3, continued		
Cuscuta gronovii	AM711639.1	AM711639.1
Dichanthelium acuminatum (Panicum acuminatum)	NC_030623.1	NC_030623.1
Dichanthelium oligosanthes var. scribnerianum (*Panicum oligosanthes* var. *scribnerianum*)	HM352804.1	HQ713384.1
Elaeagnus angustifolia	NC_040992.1	NC_040992.1
Eleocharis acicularis	KJ513595.1	AB369953.1
Eleocharis compressa	MK520032.1	MG228062.1
Eleocharis elliptica	MK520033.1	MG228117.1
Eleocharis erythropoda	HQ593278.1	HQ590075.1
Eleocharis geniculata (*Scirpus validus*)	JQ587359.1	AM999823.1
Eleocharis rostellata	None	AM999828.1
Elymus virginicus	HM352812.1	KC237165.1
Epilobium ciliatum	MG220876.1	KP643880.1
Epilobium coloratum	None	HQ590077.1
Epilobium leptophyllum	MG220679.1	MG246521.1
Equisetum arvense	JN968380.1	NC_014699.1
Erechtites hieraciifolius	MH621597.1	KJ773481.1
Erigeron annuus	MH659622.1	MH203367.1
Erigeron philadelphicus	KP175072.1	JX848412.1
Eriophorum angustifolium	KJ513597.1	JX644681.1
Eupatorium perfoliatum	MG225005.1	EU676925.1
Euthamia graminifolia	KP175079.1	MN601448.1
Eutrochium maculatum	MK520079.1	EU676922.1
Filipendula ulmaria	MH593730.1	KM360786.1
Fragaria virginiana	NC_019602.1	NC_019602.1
Frangula alnus (*Rhamnus frangula*)	AY257532.1	KM360790.1
Fraxinus americana	NC_042449.1	NC_042449.1
Fraxinus pennsylvanica	NC_043874.1	NC_043874.1
Galium asprellum	MG225302	KJ593429
Galium boreale	HQ593306.1	JX848534.1
Galium obtusum	MK520124.1	MG223213.1
Galium tinctorium	MK520125.1	KJ773532.1
Galium trifidum	MK520126.1	MG906212.1
Galium triflorum	MG225149.1	KJ841344.1
Gentianopsis crinita	MK520135.1	MG224030.1
Gentianopsis virgata (*Gentiana procera*)	MK520136.1	MG221488.1
Geranium maculatum	KP642821.1	KP644002.1
Geum canadense	MG221024.1	DQ006121.1
Geum laciniatum	MK520140.1	MG248293.1

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Table S3, continued		
Geum laciniatum	MK520140.1	MG248293.1
Glechoma hederacea	HQ593314.1	L14292.1
Glyceria striata	KC123408.1	KC123366.1
Helenium autumnale	MG225340.1	GU817764.1
Helianthus grosseserratus	MK520166.1	MG222217.1
Heliopsis helianthoides var. *occidentalis*	KT176590.1	KT178112.1
Heracleum maximum	KX677754	MG224668
Hesperis matronalis	NC_035511.1	NC_035511.1
Hierochloe odorata (*Anthoxanthum hirtum*)	KC474037.1	KC481966.1
Humulus lupulus	NC_028032.1	NC_028032.1
Hydrophyllum virginianum	HQ593327.1	HQ590137.1
Hypericum perforatum	AB698447.1	HQ332081.1
Hypericum sphaerocarpum	None	MK526004.1
Hypoxis hirsuta	MK520201.1	KP644117.1
Impatiens capensis	KT176615.1	KT178138.1
Iris pseudacorus	MK593164.1	MK593164.1
Iris virginica	KC118951.1	HQ182432.1
Juncus dudleyi	KX676959.1	HQ590144.1
Juncus torreyi	MG216975.1	HQ590148.1
Laportea canadensis	AY257537.1	AF500356.1
Lathyrus palustris	NC_027078.1	NC_027078.1
Leersia oryzoides	FN908060.1	FN870396.1
Lemna minor	NC_010109.1	NC_010109.1
Ligustrum vulgare	NC_042274.1	NC_042274.1
Liparis loeselii	MF374688.1	MF374688.1
Lobelia kalmii	KX676934.1	DQ356166.1
Lobelia siphilitica	MG225330.1	DQ006102.1
Lonicera x bella	KJ840934.1	KJ841386.1
Lycopodioides apoda (Selaginella apoda)	None	KY023315.1
Lycopus americanus	MG224798.1	HQ590170.1
Lycopus europaeus	AY840154.1	HM850150.1
Lycopus uniflorus	KX677644.1	KJ841399.1
Lycopus virginicus	MF350270.1	KJ773663.1
Lysimachia ciliata	MG950437.1	MG950543.1
Lysimachia nummularia	JN895958.1	KM360869.1
Lysimachia quadriflora	MG950499.1	MG950605.1
Lysimachia thyrsiflora	MK926103.1	MG950616.1
Lythrum alatum	MK520296.1	KJ773667.1

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Table S3, continued		
Lythrum salicaria	NC_042891.1	NC_042891.1
Maianthemum racemosum (*Smilacina racemosa*)	EU850269.1	AY149376.1
Maianthemum stellatum (*Smilacina stellata*)	EU850270.1	JQ273928.1
Mentha canadensis (*Mentha arvensis* var. *villosa*)	NC_044082.1	NC_044082.1
Micranthes pensylvanica	MK520316.1	MG249321.1
Mimulus ringens	MF350104.1	MF349630.1
Monarda fistulosa	GU381745.1	MN601458.1
Muhlenbergia glomerata	KU530246.1	MG227648.1
Muhlenbergia mexicana	MG217072.1	KJ841423.1
Myosotis verna	MK520340.1	MK526219.1
Nasturtium officinale	NC_009275.1	NC_009275.1
Nothoscordum bivalve (*Allium canadense*)	JX903549.1	JX903138.1
Onoclea sensibilis	NC_035860.1	NC_035860.1
Oxalis stricta	AY935936.1	KC481641.1
Oxypolis rigidior	MK520377.1	MG224568.1
Packera paupercula	JN966397.1	MG222241.1
Parnassia glauca	AY935908.1	AY935729.1
Parthenocissus inserta	HQ593380.1	KM360918.1
Parthenocissus quinquefolia	JQ844151.1	AJ402985.1
Pedicularis lanceolata	HG423968.1	MG222671.1
Penthorum sedoides	EF179063.1	L11197.2
Persicaria amphibia (*Polygonum coccineum*)	KY978013.1	KM360922.1
Persicaria hydropiper	KY978021.1	AB008781.1
Persicaria hydropiperoides (*Polygonum hydropiperoides*)	KY978022.1	HM850244.1
Persicaria virginiana	KY978012.1	EF653775.1
Phalaris arundinacea	KU883572.1	AJ784827.1
Phragmites australis	NC_022958.1	NC_022958.1
Pilea pumila	KF138046.1	U00438.1
Poa nemoralis (Agrostis alba)	KY562592.1	KY562592.1
Poa pratensis	MF999127.1	KX513081.1
Populus deltoides	NC_040929.1	NC_040929.1
Populus tremuloides	KJ840971.1	JX848535.1
Primula meadia (Dodecatheon meadia)	AY647482.1	MK525718.1
Prunus virginiana	AF288118.1	KT458055.1
Pycnanthemum virginianum	MG225271.1	MG224042.1
Ranunculus septentrionalis	FM242768	None
Ratibida pinnata	MF350172.1	KX702303.1
Rhamnus cathartica	AY257533.1	KM360955.1

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Table S3, continued

Rhynchospora fusca (*Rhynchospora capillacea*)	MK520526	AM999859
Ribes americanum	HQ593412.1	JX848471.1
Rosa blanda	MG221046.1	HQ590245.1
Rosa multiflora	NC_039989.1	NC_039989.1
Rubus occidentalis	EU749386.1	EU676994.1
Rudbeckia hirta	AY215856.1	MN601467.1
Rudbeckia laciniata var. laciniata	MN518844.1	MN518844.1
Rumex crispus	MN564930.1	MN564930.1
Rumex orbiculatus	MK520577.1	MK526577.1
Sagittaria latifolia	HQ456467.1	LC541695.1
Salix bebbiana	EU790689.1	AB012783.1
Salix discolor	EU790693.1	FJ788568.1
Salix eriocephala	EU790694.1	EU677001.1
Salix interior	NC_024681.1	NC_024681.1
Salix myricoides (*Salix glaucophylloides*)	KM002260.1	KX016454.1
Salix nigra	KM002266.1	AB012790.1
Salix petiolaris	KM002281.1	KJ841546.1
Sambucus canadensis	MF350157.1	KP088849.1
Schizachyrium scoparium (*Andropogon scoparius*)	MF170217.1	MF170217.1
Schoenoplectus acutus var. *acutus (Scirpus acutus)*	KX036981.1	KX036970.1
Schoenoplectus tabernaemontani	LT900080.1	KT626775.1
Scirpus atrovirens	KJ513630.1	HQ590264.1
Scirpus cyperinus	KJ513631.1	EF178580.1
Scleria verticillate	KX369540.1	KJ773874
Scrophularia marilandica	MK520633.1	MG222163.1
Scutellaria galericulata (*Scutellaria epilobiifolia*)	JN895418.1	Z37459.1
Scutellaria lateriflora	HQ593438.1	HQ590266.1
Setaria faberi	KF163775.1	KF163542.1
Silphium perfoliatum	AY215859.1	AY215176.1
Smilax tamnoides	KP643053.1	KJ773905.1
Solanum dulcamara	NC_035724.1	NC_035724.1
Solidago canadensis	MF159464.1	KM360988.1
Solidago canadensis var. scabra	EU749409.1	EU677019.1
Solidago gigantea	HQ593451.1	HM850369.1
Solidago nemoralis	EU749420.1	MN601473.1
Solidago ohioensis	MK520681.1	MG224454.1
Solidago patula	MK520682.1	MG224171.1
Solidago riddellii	MK520683.1	MG223782.1

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Solidago uliginosa	MG224992.1	MG224513.1
Sorghastrum nutans	NC_030498.1	NC_030498.1
Sparganium eurycarpum	HQ180886.1	HQ182450.1
Sphenopholis intermedia	KX873520.1	MG226614.1
Sphenopholis obtusata	LN554455.1	KJ773923.1
Spiranthes cernua	MF286465.1	AF074229.1
Spiranthes ovalis var. erostellata	MG755178.1	MG228052.1
Sporobolus michauxianus (*Spartina pectinata*)	MF597618.1	AJ784821.1
Stachys pilosa	HQ911559.1	JX848474.1
Stachys tenuifolia	MK520711	MK526752
Strophostyles helvola	DQ443469.1	KX385994.1
Symphyotrichum firmum (*Aster puniceus firmus*)	None	MG224649.1
Symphyotrichum lateriflorum (*Aster lateriflorus*)	EU749437.1	EU677047.1
Symphyotrichum novae-angliae (*Aster novae-angliae*)	EU749440.1	GU817740.1
Symphyotrichum novi belgii	KP175102.1	KF997334.1
Symphyotrichum ontarionis (*Aster ontarionis*)	MK520732.1	KJ593707.1
Symphyotrichum oolentangiense (*Aster azureus*)	MK520733.1	MG221284.1
Symphyotrichum pilosum	EU749444.1	EU677053.1
Symphyotrichum puniceum	HQ593461.1	HQ590290.1
Symplocarpus foetidus	AM920551.1	L10247.2
Taraxacum officinale	NC_030772.1	NC_030772.1
Teucrium canadense	KT176608.1	KT178131.1
Thalictrum dasycarpum	MG221041.1	JX258350.1
Thalictrum revolutum	MK520749.1	JX258393.1
Thelypteris confluens (Dryopteris thelypteris)	None	KT626846
Thelypteris palustris var. pubescens (Dryopteris thelypteris var. pubescens)	JF832292.1-UNVERIFIED	HQ676513.1
Toxicodendron radicans (*Rhus radicans*)	AY594491.1	HQ590304.1
Triadenum virginicum var. *fraseri* (*Hypericum virginicum fraseri*)	HQ331688.1	JX664051.1
Triglochin maritima	AB088782.1	KM361021.1
Typha angustifolia	NC_050678.1	NC_050678.1
Typha latifolia	NC_013823.1	NC_013823.1
Ulmus pumila	NC_032721.1	NC_032721.1
Verbena hastata	KT176612.1	KT178132.1
Verbesina alternifolia	MK520797.1	MG222952.1
Viburnum lentago	HQ593493.1	EU677069.1
Viburnum opulus	MG225093.1	KP088910.1
Viburnum prunifolium	MF350199.1	KP088911.1

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Viola cucullate	HQ593502.1	HQ590335.1
Viola macloskeyi subsp. pallens	HQ593503.1	HQ590336.1
Viola nephrophylla	MK520805.1	MG247710.1
Viola pubescens	HQ593504.1	JX664075.1
Viola sororia	HQ593506.1	HQ590339.1
Vitis riparia	NC_039680.1	NC_039680.1
Xanthium strumarium var. canadense	MH070594.1	KX214126.1

Reference

World Flora Online, http://www.worldfloraonline.org/, accessed most recently, 24 Nov. 2021.