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Plant colonization of a restored wetland in northern Kentucky: Contribution of seeding vs. natural sources[a]

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Abstract. Despite their important ecosystem services, most wetlands in Kentucky and the surrounding region have been lost. Many restoration attempts have occurred, but the success of seeding or planting, which is often done after restoration, is unclear. To our knowledge, seeding success relative to other propagule inputs has never been quantified. We measured the restoration success of a four-year-old emergent open-canopy wetland, with seven adjacent ponds near the banks of the Ohio River in Kentucky. Potential restoration plant origins include (a) from the seedbank and surrounding area, (b) a native seed mix designed for upland and wetland restorations, and (c) from the onsite prerestoration wetland. We tested the hypotheses that elevation and distance from an established wetland pond are driving factors to establish species that dominate the wetland vegetative cover. Plant cover and relative elevation were determined in 78 1-m^2 plots. Although most species (73%) came either from seedbank or offsite sources, the remainder appear to have come from seeding (14–16%) and the prerestoration wetland (11–14%), and the latter two sources accounted for almost 50% of the total cover at the site. Ordination supported the hypotheses that distance from the prerestoration pond and relative elevation were the two most important factors determining patterns of plant cover. Despite its modest contribution to plant diversity, the prerestoration pond had an important effect on plant patterns. While the site falls into Kentucky Wetland Rapid Assessment (KY-WRAM) Category 1 (most disturbed), prevalence index (PI) scores, which are based on species wetland classifications, appear to be similar to or higher than those of other created wetlands in the United States.

Key words: coefficient of conservatism, invasive plants, NMDS, nonnative plants, Ohio River

While the array of ecosystem services offered by wetlands is now well appreciated, over half of all wetlands in the contiguous United States have been lost (Vileisis 1997). Mitsch and Day (2006) estimated that 80–90% of the original wetlands in the Midwest have been lost, while Dahl (2011) estimated that > 80% of Kentucky wetlands have been lost. Wetlands in the Mississippi-Ohio-Missouri River Basin are critical for ameliorating eutrophication in the Gulf of Mexico. Thus, wetland restoration is clearly required, especially in the central United States, and particularly in Kentucky.

There are, however, many challenges in wetland restoration, and Mitsch *et al*. (2012) have questioned the effectiveness of many restoration projects. Restored wetlands often have lower diversity and more nonnative species than intact wetlands (Seabloom and van der Valk 2003, Balcombe *et al*. 2005, Spieles 2005, Matthews and Spyreas 2010). While some assessment has been done in Ohio (*e.g.,* Mitsch and Day 2006, Mitsch *et al*. 2012) and West Virginia (Balcombe *et al*. 2005), little assessment of restored wetlands has been done in Kentucky, especially within Ohio River Basin watersheds (*e.g.,* Reeder 2011).

Several methods have been applied to measure wetland restoration success based on vegetation. Most use a coefficient of conservatism (CC) for each species that is present as a weighting factor in calculating the quality of a site. Coefficients of conservatism indicate the sensitivity of a plant to disturbance and its fidelity to a particular habitat, usually wetlands, and range from 0 to 10 (Swink and Wilhelm 1994). Although CCs have an element of subjectivity, they can be applied objectively in the region for which they have been developed. Both the mean of the coefficients of conservatism (meanCC) and the Floristic Quality Assessment Index (FQAI) have been used to assess wetland quality (*e.g.,* Andreas *et al*. 2004, Smith 2016), and as Andreas *et al*. have shown that both measures usually covary, we have decided to use the meanCC. One drawback of these measures is that traditionally they do not adjust for nonnative species, which should lower the value of the index used. However, Miller and Wardrop (2006) have proposed a modified index that incorporates nonnative species, resulting in

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lower values. An equivalent method is to assign a conservation of conservatism value of 0 to nonnative plants and include them in the calculations (Smith 2016).

The U.S. Environmental Protection Agency has proposed a three-level system—landscape, rapid, and intensive—for wetland assessment, where indicators of human disturbance are used to gauge ecosystem quality (US EPA 2006). Kentucky uses the Kentucky Wetland Rapid Assessment Method (KY-WRAM; Kentucky Division of Water 2019) across the state, but until recently there was no intensive level-three Vegetative Index of Biotic Integrity (VIBI) to validate the KY-WRAM. Smith (2016) developed a VIBI and successfully tested its effectiveness in discriminating among KY-WRAM categories, from category one (most disturbed) to category three (least disturbed), for a large part of the state, including northern Kentucky. This VIBI uses two metrics, meanCC (including nonnative plants) and the absolute cover of nonnative species, which are each divided into five categories that are scored 1 to 5; the resulting VIBI score ranges from 2 to 10.

Restoration without intervention beyond altering the hydrology of the wetland by, for example, excavation and channeling, often requires a long period before full functionality is regained, and so planting, either of seed or rootstock, is often required (Meyer *et al*. 2010). Nonetheless, many of the species that establish in a restored wetland will come from the surrounding area and/or the seedbank without intervention. The authentication and importance of propagule sources, either natural or artificial, to the reestablishment of a wetland has rarely been addressed directly, and one of our objectives was to show how this might be done. Thus, while the overall purpose of this study was to assess the restoration success of an emergent open-canopy wetland near the banks of the Ohio River in Kentucky four years after construction and artificial seeding, we were also interested in the relative contributions of propagule sources, especially seedling *vs*. seedbank/offsite sources, in revegetation. We also attempted to determine which factors determined the presence and cover of plant species in various locations in the wetland. Nonintervention plants from a nearby source could readily establish themselves in a newly created wetland habitat. Similarly, the more diverse the topography, or, in the case of a wetlands, the elevation, the greater the abundance of species that could dominate plant cover in a restored wetland. Therefore, we hypothesize that the distance from a nonintervention source of plants and the elevational topography of the site are the two main factors driving plant cover dominance in a restored wetland. The established baseline from this study will be a foundation for future investigations in these wetlands, when we initiate a manipulative experiment evaluating the removal of nonnative species for this area. In addition, our findings may help guide restoration efforts for creating high quality wetlands.

Materials and Methods. SITE. The study area is part of the St. Anne Wetlands and Woods (39°2′4″N, 84°22′24″W) in Melbourne, Kentucky, USA. This 40-ha property, mainly bottomland forest wetland, is located near the Ohio River and supports an extensive system of ephemeral ponds and streams. It is owned and administered by the Campbell County Conservation District. Braun (1916) studied this area (which she referred to as the Melbourne Forest) and characterized it as a floodplain forest of the depression forest subtype. Forest composition in this area was studied more recently by Bryant (1987) and Bryant and Held (2004), and Boyce *et al*. (2012) studied the effects of the invasive *Lonicera maackii* on transpiration and hydrology.

The restored emergent wetland ($\sim$ 0.86 ha) that is the subject of this study is part the Wetland and Woods and is adjacent to the Northern Kentucky University Research and Education Field Station (NKU REFS). It lies south of the Ohio River and north of a railroad line, within in the 100-year floodplain of the Ohio River (flooding did not occur between the initial restoration and this study). It was used for agriculture and had become an old field before restoration. One pond (Pond 1) had formed at the east end of the area before restoration and was regarded as a potential propagule source. The restoration occurred in September 2012. Existing vegetation was removed, and six more ponds were dug with earthmoving equipment, following procedures described in Biebighauser (2007). Straw was placed as cover, and winter rye (*Secale cereal*; nomenclature follows USDA NRCS 2019) was broadcast-planted as a winter crop to prevent erosion. Two native seed mixes, one for lowlands and the other for uplands, were also broadcast-applied in combination by the Northern Kentucky

Table 1. Species in two native seed mixes applied to the restored wetlands at St. Anne Woods and Wetlands by the Northern Kentucky University Center for Environmental Restoration, September 7–8, 2012. Seed mixes are prepared with lots purchased from local growers and are used in area restoration projects. Amounts are in g, rounded to nearest 5 g. Nomenclature follows U.S. Department of Agriculture-Natural Resources Conservation Service (2019). Species in boldface were found in our sampling plots.

Lowland area seed mix			Upland area seed mix		
Species name	Amount	Habitat indicator	Species name	Amount	Habitat indicator
Graminoids			Graminoids		
Carex frankii	910	OBL	***Andropogon gerardii***	230	FAC
Carex scoparia	45	FACW	*Bouteloua curtipendula*	230	UPL
Carex vulpinoidea	90	OBL	*Chasmanthium latifolium*	115	FACU
Chasmanthium latifolium	115	FACU	***Dichanthelium clandestinum***	115	FAC+
Cinna arundinacea	45	FACW	*Elymus canadensis*	230	FAC−
Cyperus strigosus	25	FACW	*Elymus hystrix*	45	UPL
Dichanthelium clandestinum	115	FAC+	*Elymus villosus*	115	FACU−
Elymus riparius	680	FACW	***Elymus virginicus***	230	FACW−
Elymus virginicus	455	FACW−	*Panicum anceps*	130	FAC
Juncus torreyi	15	FACW	***Panicum virgatum***	115	FAC
Leersia oryzoides	115	OBL	*Schizachyrium scoparium*	910	FACU−
Scirpus atrovirens	5	OBL	*Sorghastrum nutans*	230	UPL
Scirpus cyperinus	455	FACW+	*Tridens flavus*	230	FACU
Spartina pectinata	45	OBL			
Forbs			Forbs		
Boehmeria cylindrica	45	FACW+	***Desmanthus illinoensis***	15	FAC
Epilobium coloratum	10	FACW+	*Echinacea purpurea*	115	UPL
Eupatorium perfoliatum	230	FACW+	*Heliopsis helianthoides*	115	UPL
Eutrochium fistulosum	25	FACW	***Monarda fistulosa***	115	UPL
Helenium autumnale	10	FACW+	*Oenothera biennis*	115	FACU−
Helianthus tuberosus	10	FAC	*Ratibida pinnata*	455	UPL
Hibiscus moscheutos	250	OBL	*Rudbeckia hirta*	230	FACU−
Ludwigia alternifolia	10	FACW+	*Rudbeckia triloba*	25	FACU
Penstemon digitalis	10	FAC	*Silphium perfoliatum*	45	FACU
Penthorum sedoides	10	OBL	*Silphium trifoliatum*	45	UPL
Senna marilandica	25	FAC+			
Symphyotrichum novae-angliae	10	FAC			
Verbena hastata	135	FACW+			
Woody			Woody		
Amorpha fruticosa	45	FACW	*Hypericum prolificum*	45	FACU
Cephalanthus occidentalis	115	OBL	*Rhus glabra*	115	UPL
Platanus occidentalis	25	FACW−			

University Center for Environmental Restoration; species and amount of seed are listed in Table 1. Seed mixes, which are used by the Center for area restoration projects, are prepared with lots purchased from local growers; information on possible contamination was not available. Pond locations, approximate surface areas, and distances from Pond 1 are shown in Fig. 1. All ponds are fed and drained by groundwater most of the time (*i.e.*, they are not fed or drained by streams) and they often experience hydraulic lift when the Ohio River is high (R. Boyce and R. Durtsche, personal observations). Pond maximum depths rarely exceed $\sim$ 1 m. Ponds 4 and 5 are connected during periods of high water. While large areas of all seven ponds are exposed during dry periods, only Pond 3 has been observed to dry completely since its construction.

Measurements. A weather station was established in 2014 to measure water. Hourly measurements of water depth, made with a Campbell Scientific Instruments CS-451 submersible pressure transducer (Campbell Scientific, Logan, UT), from September 26, 2014, to October 17, 2016, were used to determine mean depth of a single location in Pond 6. To determine sampling plot elevations, a snapshot assessment was taken on October 17, 2016; plot elevations relative to the Pond 6 water level on that day were determined using a survey rod, clinometer, and a meter tape, and they were then adjusted to reflect relative

FIG. 1. Aerial photo of restored pond area at St. Anne Woods and Wetlands. Parcel owned by the Campbell County Conversation District is outlined in green. Brown area at top is the Ohio River, with a railroad at the bottom. The seven ponds are outlined and numbered from right to left in black. Approximate area of the restored area is 0.86 ha. Approximate pond areas: Pond 1 = 370 m^2; Pond 2 = 660 m^2; Pond 2a = 140 m^2; Pond 3 = 100 m^2; Pond 4 = 430 m^2, Pond 5 = 715 m^2; and Pond 6 = 365 m^2. Pond 2a was not included in this study. Photograph and areas were obtained from LINK-GIS (2016). Distances of each pond center from the center of Pond 1: Pond 2 = 55.5 m; Pond 3 = 60.0 m; Pond 4 = 95.5 m; Pond 5 = 129.5 m; and Pond 6 = 173.8 m.

elevations above or below the 2-year mean water depth of Pond 6. Water levels of all ponds were assumed to be the same.

Transects were laid down in 2016, each starting near the center of six of the seven ponds at the site. Four transects ran in each of the cardinal directions from this center point, with a flag placed every 5 m along each transect, until the forest edge or shrub thickets (consisting of *Rosa multiflora* and/or *Rubus allegheniensis*) were encountered (ca. 5–35 m). A 1-m × 1-m sampling frame was centered on each flag on each transect to form a sampling plot, and the vegetative canopy cover of each species was estimated using the Pfister scale (Pfister *et al*. 1977), with coverage classified as T (trace) = 0–1%, 1 = 1–5%, 2 = 5–25%, 3 = 25–50%, 4 = 50–75%, 5 = 75–95%, or 6 = 95–100%. Species nomenclature follows the USDA PLANTS Database (USDA NRCS 2019). Plots were surveyed three times, in May (spring), July (summer), and September (fall) 2016. A total of 78 plots were surveyed; five other plots were not sampled because they were in water too deep to contain any emergent vegetation. The number of plots varied from 10 to 17 per pond. For each species in each plot, the midpoint percent cover value from the maximum calculated cover score from the three sampling dates was used to calculate total plot cover. Maximum nonnative plant cover and the fraction of total maximum plant cover made up of nonnative plants was also determined for each plot.

A mean wetland indicator status (WIS) score for each plot was determined, using the WIS classification given in Jones (2005) and USDA NRSC (2019) for each species present (if available). A numerical value was assigned to each species' classification; that is, UPL (obligate upland) = 1, FACU (facultative upland) = 2, FAC (facultative) = 3, FACW (facultative wetland) = 4, and OBL (obligate wetland) = 5, and used to calculate a mean WIS score for each plot. The prevalence index (PI), which is a version of the mean WIS score weighted by plant species cover, was also calculated for the site as a whole (Wentworth *et al*. 1998). A meanCC for the entire wetland site and for each plot was calculated as meanCC = $\Sigma(CC_i)/N$, where CC_i is the coefficient of conservatism for species i and N is the total number of all species present, using the coefficients of conservatism for native plants that were developed for Kentucky by Gianopulos (2014). A few species found at our site were not evaluated by Gianopulos (*i.e*., *Desmodium paniculatum*, *Geranium carolinianum*, *Monarda fistulosa*, and *Sisyrinchium montanum*); in these cases, values from Ohio were used (Andreas *et al*. 2004). Values of 0 were assigned to nonnative plants. The Vegetative Index of Biotic Integrity (VIBI) for the entire wetland site was calculated using the method described in Smith (2016), which uses five categories of meanCC and five of absolute total nonnative plant cover to calculate a score ranging from 2 (lowest quality) to 10 (highest quality).

ANALYSIS. Nonmetric multidimensional scaling (NMDS) was applied to the vegetative cover data in R (R Core Team 2019), using the package labdsv. Two axes were specified. Surface contours for two factors, the relative elevations and sampling plots distance from the center of Pond 1, were fit to the two axes of the NMDS results, using a general additive model (GAM) with Gaussian error distributions for relative elevation and distance from the center of Pond 1. These factors were chosen because elevation was expected to have a large effect on species composition,

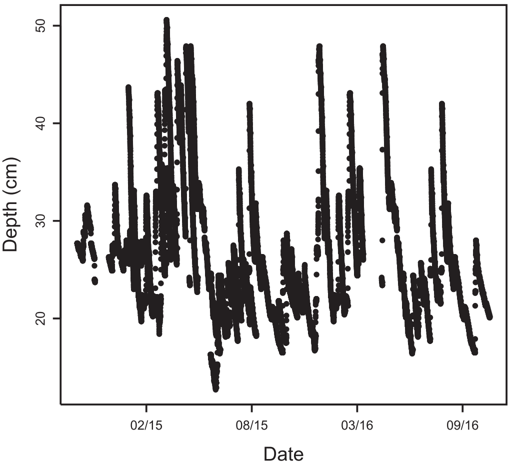

FIG. 2. Hourly water levels measured at Pond 6, from September 26, 2014 to November 17, 2016. Gaps denote missing data. Water depth on October 17, 2016 was 24.1 cm. Median and mean depths were 23.4 and 25.2 cm, respectively, with a standard deviation of 6.40 cm.

and Pond 1 was considered to be a source of naturally dispersed wetland species. Based on surface contours, Spearman rank correlations were calculated between plot values of these two factors and the NMDS scores for each axis. We used Spearman rank correlation (r_s; Zar 2010) throughout, because many of our factors had nonnormal distributions (data not shown). We ran bootstrap estimates of r_s to validate analyses where large numbers of zeros might have affected the results.

Pond 1 was a potential natural (nonintervention) seed source. Thus, plots closer to the center of Pond 1 were hypothesized to have higher values of meanCC, and these indices were correlated against the NMDS axes and pond number. Because elevation determines hydrologic regimes in wetlands, we correlated several factors against elevation, including maximum plant cover, nonnative plant cover, fraction of cover that was nonnative, plot WIS score, and plot meanCC scores. We then estimated the contribution of plant propagules both by species presence and by cover from three potential sources: Pond 1 (the nonintervention seed source), the applied seed mixes (intervention), and seedbank/offsite sources (a nonintervention but random/unpredictable potential seed source). All analyses used an error rate assumption of $\alpha = 0.05$.

Results. Measured water depths of Pond 6 are shown in Fig. 2. From September 2014 to November 2016, median depth at the gauge was 24.40 cm, with a mean ± standard deviation of 25.94 ± 6.65 cm, and a minimum and maximum of 12.70 and 50.60 cm, respectively. Relative elevations of all plots were determined in relationship to the mean depth of Pond 6, and the water surface levels of all ponds were assumed to be the same. Plot elevations, relative to the mean water level of Pond 6, are shown in Table 2. They ranged from 0.48 m below to 0.51 m above this elevation.

The maximum coverages of each species found in each plot across the growing season were totaled to calculate the maximum plant cover in each plot, and the mean was 144.33%, with a range of 0.50–345.00% for all species within each plot (Table 2). A total of 73 taxa were identified; five were identified only to the family or genus level, and one forb could not be identified (Table 3). The five species with the most cover were *Scirpus cyperinus, Leersia oryzoides, Lespedeza cuneata, Rubus allegheniensis*, and *Solidago rugosa*. Fourteen of the species were nonnative (18.9%), while 12 other species (16.4%) were present in the seed mixes (bolded in Table 1) that had been applied to the site at the time of restoration. The mean WIS score for the site, based on species present, was 3.5, which falls between FAC (3) and FACW (4). In contrast, the WIS score for the seed mixes were 4.0 and 2.0 for the lowland and upland seed mixes, respectively, while it was 3.8 for the 12 species in the seed mixes that we found at our site. The PI for the site, which is the WIS score weighted by species cover, was 3.7. The largest WIS categories by cover were OBL and FACW (Table 4); almost 75% of the cover was hydrophytic vegetation, as defined by the U.S. Army Corps of Engineers. The meanCC value for the site was 3.31, and the absolute nonnative cover was 30.1%; this resulted in a VIBI score of 1 for meanCC (0–3.38) plus 1 for absolute cover of nonnatives (> 0.57%), for a total of 2 (Smith 2016), placing the site in KY-WRAM Category 1 (most disturbed) for emergent wetlands.

The NMDS results showed that the first axis was aligned with site distance from the center of Pond 1, with plots near Pond 1 with positive values and those near Pond 6 with negative ones (Fig. 3), while the second axis was aligned with plot elevation, with highest elevations with positive values and lowest with negative ones. The proportions of deviance explained from the surface fit GAMs, which are similar to correlation

Table 2. Elevation of each sampling plot relative to mean water level in Pond 6 from DOY 268 in 2014 to DOY 321 in 2016 (25.9 cm). Plot designator: First number is pond number, letter refers to direction from pond center (C = center), last digit(s) refer to distance from pond center in m. Distance from 1.C.0 is the distance of the plot from the center of Pond 1. Maximum cover is the midpoint percent cover value from the maximum calculated cover score from the three sampling dates.

Plot	Relative elevation (m)	Distance from 1.C.0 (m)	Maximum cover (%)
1.C.0	0.09	0.0	122.5
1.W.5	0.05	5.0	121.5
1.W.10	0.04	10.0	157.5
1.W.15	−0.03	15.0	120.0
1.W.20	−0.06	20.0	115.5
1.W.25	−0.05	25.0	206.5
1.N.5	0.00	5.0	87.0
1.N.10	−0.17	10.0	48.0
1.N.15	0.04	15.0	108.0
1.N.20	0.30	20.0	345.0
1.E.5	0.10	5.0	202.5
1.E.10	0.04	10.0	137.5
1.E.15	0.04	15.0	285.0
1.S.5	−0.04	5.0	135.0
1.S.10	−0.09	10.0	200.0
1.S.15	−0.09	15.0	122.5
1.S.20	0.03	20.0	69.0
2.C.0	0.23	55.5	184.5
2.W.5	−0.16	60.4	125.5
2.W.10	−0.16	65.2	113.0
2.W.15	0.10	70.1	150.5
2.N.5	−0.14	56.9	149.0
2.E.5	−0.18	50.7	119.5
2.E.10	−0.13	45.9	136.5
2.E.15	−0.19	41.1	204.0
2.E.20	0.08	36.4	168.5
2.S.5	−0.25	54.5	182.5
2.S.10	−0.10	54.0	151.5
3.C.0	−0.30	60.0	82.5
3.W.5	−0.29	64.2	128.5
3.W.10	−0.26	68.6	242.5
3.W.15	0.28	73.0	238.5
3.W.20	0.13	77.4	202.0
3.N.5	−0.09	62.9	157.5
3.E.5	−0.44	55.9	43.5
3.E.10	−0.31	52.0	222.0
3.E.15	−0.17	48.2	221.5
3.S.5	−0.14	55.1	154.5
3.S.10	0.14	57.4	178.0
4.C.0	0.14	95.5	105.0
4.W.5	−0.29	99.2	3.0
4.W.10	−0.34	102.9	3.0
4.W.15	−0.28	106.8	91.0
4.W.20	0.08	110.8	152.0
4.W.25	0.15	114.9	226.0
4.N.5	−0.28	99.0	100.5
4.E.5	−0.24	92.0	18.0
4.E.10	0.01	88.6	240.5
4.E.15	0.13	85.3	200.5
4.S.5	−0.34	92.1	41.0
4.S.10	0.16	88.9	124.5
4.S.15	0.29	85.8	152.5
5.C.0	0.00	129.5	196.0
5.W.5	−0.41	134.1	175.0
5.W.20	−0.31	148.1	62.5
5.W.25	−0.10	152.8	163.5
5.W.30	0.05	134.1	294.5
5.N.5	0.12	131.5	38.0
5.N.10	−0.26	133.7	63.0
5.N.15	−0.01	136.1	222.0
5.E.5	0.08	124.9	58.5
5.E.10	−0.36	120.4	3.0
5.E.15	−0.29	115.8	154.5
5.E.20	−0.28	111.4	142.5
5.E.25	−0.28	106.9	129.0
5.E.30	−0.19	102.6	241.0
5.E.35	−0.22	98.3	115.5
5.S.5	−0.48	127.6	65.5
6.C.0	0.08	173.8	296.5
6.W.5	−0.37	178.4	0.5
6.W.10	−0.24	182.9	21.0
6.W.15	0.09	187.5	135.0
6.W.20	0.51	192.2	265.5
6.W.25	0.39	196.8	249.5
6.N.5	0.12	175.9	178.5
6.N.10	0.28	178.2	149.0
6.E.5	−0.31	169.3	18.0
6.E.10	0.07	164.8	224.0
Mean	−0.072	103.8	144.3
Minimum	−0.48	0.0	0.5
Maximum	0.51	196.8	345.0

coefficients, were 19.6% and 45.7% for distance from the center of Pond 1 and plot elevation, respectively. The respective Spearman correlations for distance from Pond 1 and elevation and their NMDS axes were $r_s = -0.43$ ($P < 0.001$) and $r_s = 0.62$ ($P < 0.001$), respectively.

The first NMDS axis and plot meanCC were significantly positively correlated ($r_s = 0.38$, $P < 0.001$). In a similar manner, meanCC declined with distance from the center of Pond 1 ($r_s = 0.23$, $P = 0.040$). Maximum plant cover, nonnative plant cover, and the fraction of cover made up of nonnative plants were all positively correlated with plot elevations, while plot WIS scores were negatively correlated (*i.e.*, plots with more obligate wetland species were found at lower elevations; Fig. 4). Plot meanCC was also negatively correlated with the second NMDSaxis ($r_s = -0.27$, $P = 0.018$). Plot meanCC scores declined

Table 3. Mean maximum species cover (%) per sampling plot, along with number of plots in which the species was found. Nonnative species (Jones 2005) are labeled with *, while species present in the seed mixes are marked with §. Distance from Pond 1 center and relative elevations for each species are both weighted by maximum cover of species in sampling plots. Species showing evidence of propagation from Pond 1 are shown in boldface.

Species	Mean maximum cover (%)	Number of plots	Distance from Pond 1 center (m)	Relative elevation (m)
Scirpus cyperinus§	29.68	41	61.6	−0.09
Leersia oryzoides§	19.85	47	106.9	−0.10
*Lespedeza cuneata**	15.92	30	80.1	0.03
Rubus allegheniensis	8.35	26	87.6	0.14
Solidago rugosa	7.04	20	84.9	0.16
Symphyotrichum lateriflorum	6.28	32	90.4	0.01
Dichanthelium clandestinum§	6.03	27	94.7	0.02
Ludwigia palustris	5.89	31	57.4	−0.15
Desmodium paniculatum	5.68	34	75.4	0.01
*Microstegium vimineum**	4.97	7	112.4	0.14
Salix exigua	3.63	5	74.8	0.07
Hibiscus moscheutos§	2.49	9	11.6	−0.05
Alisma subcordatum	2.41	19	59.9	−0.16
*Typha angustifolia**	2.40	9	59.9	−0.27
*Lonicera japonica**	2.25	12	93.1	0.12
Acer rubrum	2.07	23	49.5	0.05
Carex caroliniana	1.81	9	143.0	0.24
Eleocharis obtusa	1.50	10	92.5	−0.25
Typha latifolia	1.44	10	116.9	−0.26
*Rosa multiflora**	1.19	4	130.5	0.20
Populus deltoides	1.09	1	20.0	0.30
*Lysimachia nummularia**	0.96	2	165.5	0.22
Juncus effusus	0.85	11	31.5	−0.01
*Glechoma hederacea**	0.75	4	69.4	0.29
Cyperus strigosus§	0.71	3	18.2	−0.01
*Polygonum cespitosum**	0.58	4	80.1	0.12
Agrimonia parviflora	0.58	3	53.3	0.13
Carex bushii	0.58	3	56.5	−0.03
Pilea pumila	0.54	6	39.5	−0.03
*Lythrum salicaria**	0.50	5	104.3	−0.06
Unidentified *Asteraceae*	0.43	4	112.4	0.09
Geranium carolinianum	0.27	3	66.4	−0.01
Carex scoparia§	0.23	2	114.3	0.08
Sisyrinchium montanum	0.23	2	179.4	0.17
Euthamia graminifolia	0.23	2	53.0	−0.11
Bidens coronata	0.21	7	123.5	−0.16
Parthenocissus quinquefolia	0.21	3	188.6	0.50
Erigeron philadelphicus	0.20	6	90.0	0.00
Panicum virgatum§	0.20	2	88.1	0.02
Acer negundo	0.19	1	134.1	0.05
Andropogon gerardii§	0.19	1	85.3	0.13
*Elaeagnus umbellata**	0.19	1	73.0	0.28
Impatiens pallida	0.19	1	192.2	0.51
Lonicera maackii*	0.19	1	20.0	0.03
Monarda fistulosa§	0.19	1	114.4	−0.28
Quercus palustris	0.19	1	134.1	0.05
Carex sp.	0.19	1	196.8	0.39
Ludwigia alternifolia§	0.15	4	155.6	0.07
Fraxinus pennsylvanica	0.12	3	110.4	0.22
Apocynum cannabinum	0.08	3	80.2	0.19
*Convolvulus arvensis**	0.08	3	120.9	0.30
Vitis cinerea	0.08	3	66.7	0.17
Acer saccharinum	0.08	2	40.1	0.07
Desmanthus illinoensis§	0.08	2	48.3	−0.16
Elymus virginicus§	0.08	2	52.7	0.22

Table 3. Continued.

Species	Mean maximum cover (%)	Number of plots	Distance from Pond 1 center (m)	Relative elevation (m)
Mimulus ringens	0.08	2	160.8	–0.10
Toxicodendron radicans	0.08	2	72.3	0.02
Allium canadense	0.05	3	173.6	0.44
Verbesina alternifolia	0.04	2	68.8	–0.21
*Celastrus orbiculalis**	0.04	1	70.1	0.10
Unidentified grass	0.04	1	136.1	0.08
Hypericum mutilum	0.04	1	129.5	0.00
Impatiens capensis	0.04	1	54.0	–0.10
Lobelia inflata	0.04	1	175.9	0.12
Polygonum virginiana	0.04	1	196.8	0.39
*Pyrus calleryana**	0.04	1	55.1	–0.14
Sagittaria latifolia	0.04	1	187.5	–0.24
Ulmus rubra	0.04	1	152.8	–0.10
***Viola* sp.**	0.04	1	20.0	0.30
Unidentified forb	0.01	2	52.8	0.09
Geum vernum	0.01	1	73.0	0.28
Impatiens sp.	0.01	1	106.9	–0.28
Vernonia gigantea	0.01	1	164.8	0.07

with elevation but not significantly ($r_s = –0.15$, $P = 0.186$).

Most species had a mean cover-weighted distance from the center of Pond 1 near 100 m (Table 3), meaning they were either distributed fairly evenly across the ponds, which span a distance of ~ 200 m, or were found or aggregated near the center of the site. Certain species, however, showed high maximum covers at Pond 1, with a decline at farther distances (Fig. 5A; *i.e.*, *Acer rubrum*, *Alisma subcordatum*, *Juncus effusus*, and *Ludwigia palustris*). A number of other species were found exclusively at relatively short distances from the center of Pond 1 (Fig. 5B; *i.e.*, *Cyperus strigosus*, *Hibiscus moscheutos*, *Lonicera maackii*, *Pilea pumila*, *Populus deltoides*, and *Viola* sp.). The first two of these species were in the seed mixes (*C. strigosus* and *H. moscheutos*) but still could have been contributed from Pond 1 as well. As shown in Table 5, most of the species we found originated from seedbank/offsite sources, with < 30% of species coming from the seed mixes or Pond 1. However, about half the plant cover of the site could be attributed to the seed mixes and Pond 1.

Table 4. Percentage of maximum coverage by WIS (wetland indicator status) category and percentage classified as hydrophytic vegetation.

WIS category[a]	% Maximum coverage
OBL	47.1
FACW	15.0
FAC	12.3
FACU	13.4
UPL	11.2
Unclassified	1.0
% Hydrophytic vegetation[b]	74.4

[a] OBL = obligate, FACW = facultative wetland, FAC = facultative, FACU = facultative upland, and UPL = upland.

[b] As defined by the U.S. Army Corps of Engineers (1987): percent OBL, FACW, FAC+, and FAC.

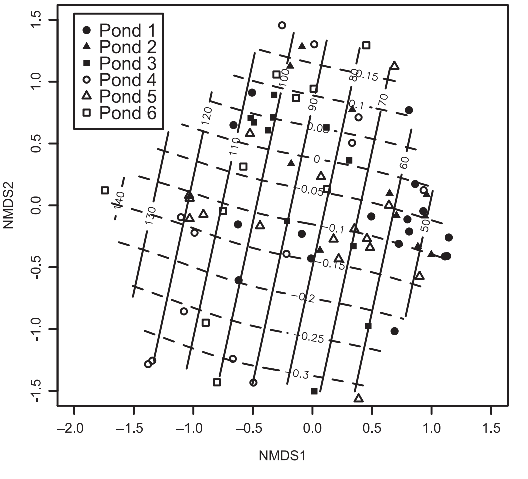

FIG. 3. Results of nonmetric multidimensional scaling, showing surface contours for distance of plots from the center of Pond 1 (solid lines) and relative elevation (m; dashed lines) above mean Pond 6 depth. Distance from the center of Pond 1 is correlated with the first axis ($r_s = –0.43$, $P < 0.001$), and relative plot elevation is correlated with the second axis ($r_s = 0.62$, $P < 0.001$). Stress value = 21.08.

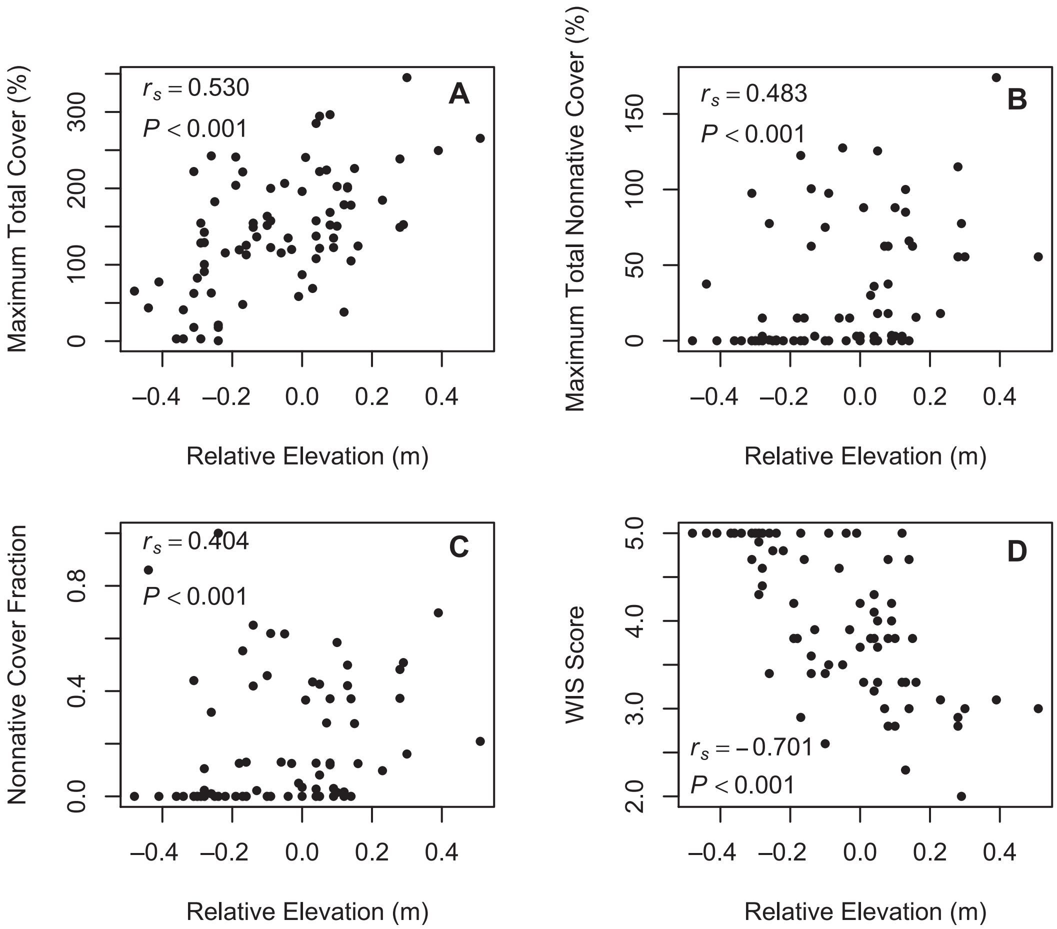

FIG. 4. Spearman rank correlations between relative plot elevations and (A) total maximum plant cover, (B) total nonnative plant cover, (C) fraction of cover made up of nonnative plants, and (D) WIS (wetland indicator status) plot scores.

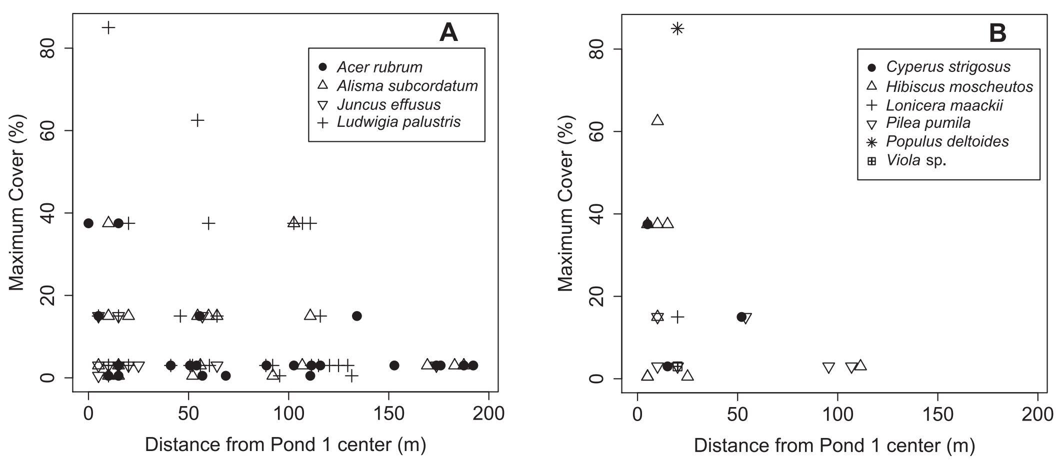

FIG. 5. (A) Plant species showing a decline in cover with distance from the center of Pond 1. (B) Plant species confined to distances close to the center of Pond 1.

Table 5. Percent contribution to species pool from various locations. Two species (*Cyperus strigosum* and *Hibiscus moscheutos*) that were in the seed mixes also show evidence of propagating from Pond 1. Numbers in parentheses show the values obtained if these species came only from Pond 1.

	Seeding	Pond 1	Offsite/seedbank
Number of species	16.4 (13.7)	11.0 (13.7)	72.6
Cover	41.8 (39.6)	9.1 (11.4)	49.0

Discussion. Overall, we deem this wetland restoration to be a mixed success in terms of plant cover. One the one hand, using the techniques developed by Smith (2016), the VIBI score was only 2, which places it in KY-WRAM Category 1 (most disturbed) for emergent wetlands. Smith (2016) noted that the median VIBI score of Kentucky emergent wetlands in her study fell below 4, much lower than those of forested and shrub wetlands, which she attributed to a history of extreme anthropogenic disturbance and proximity to agricultural lands for most emergent wetlands in Kentucky. The majority of species we found at our site, both by number of species and cover, came from either offsite or were in the seedbank (Table 5), which is consistent with a strong anthropogenic influence.

On the other hand, our site appears to be similar to the mitigated and created wetlands that have been studied in the region. For 11 mitigated wetlands in West Virginia, Balcombe *et al*. (2005) found cover averages of 43.8% OBL, 34.7% FACW, 7.1% FAC, 11.0% FACU, and 3.4% UPL, with a total of 83.8% cover considered to be hydrophytic vegetation; this would give a mean PI of 4.0. By comparison, our study had 76.6% hydrophytic vegetation cover and a mean PI of 3.7 (Table 4). We had considerably more UPL cover (11.2%) than seen by Balcombe *et al*. (2005), almost all of which was the nonnative *Lespedeza cuneata*. Spieles (2005), in a study that drew on a variety of wetland banks (*e.g*., restored or created wetlands) throughout the United States, found that for created riverine wetlands, which is the category most like our site, mean PI score was $\sim$ 2.4, nonnative plant presence was $\sim$ 22%, and the number of species/10 m^2, estimated by species accumulation curves, was $\sim$ 34. By comparison, our PI was much higher (3.7), our nonnative plant presence was similar (19%), and the number of species/10 m^2, estimated by the procedure specaccum in the package vegan in R (R Core Team 2019), was also similar (31). Thus, our site appears to be similar to created wetlands in the United States in many ways, but with a higher PI. This may be because two of the species with the most cover (*Scripus cyperinus* and *Leersia oryzoides*) were part of the seed mix applied to the site and have high WIS scores.

As noted above, *Lespedeza cuneata* was the major contributor to the higher than usual UPL score seen in Table 4. This species was originally introduced to the United States for erosion control and as a pasture crop (Tu *et al*. 2002). As our study site was in agricultural use, it is possible that it was intentionally introduced, but it is more likely that it floated in during a flooding event. We plan to control this species and other nonnative species in the future, as Matthews and Spyreas (2010) have shown that on a timescale of 5–11 years, nonnative plants often cause restored wetlands to diverge from high-quality wetlands.

Most of the species we tallied came from seedbank or offsite sources, accounting for 14 of the 18 most commonly occurring species and 7 of the 10 most abundant species (Table 5). While our site's location in the floodplain of the Ohio River means offsite propagules can drift in during floods, flooding did not occur between pond construction and this study, so many in this group of species would have already been present in the seedbank, although wind-propagated species may have blown in from elsewhere. Overall, these sources represent 72.6% of the species and 49.0% of the cover. Thus, even in restored wetlands, the major contributor propagules is from seedbank and/or offsite sources. Spieles (2005) has shown that offsite sources are very important in riverine created wetlands, as flooding brings in many propagules. Of the 52 species that were introduced by seeding, 12 were found in study plots, and 3 were the most abundant (Table 3) and frequent (data not shown) species sampled. In a tallgrass prairie restoration study that included wet meadows, Henry *et al*. (2019) estimated that 10–17 2-m $\times$ 2-m plots were sufficient to tally at least 95% of all species present. If their findings are applicable to restored wetlands, we should thus have tallied most species. By comparison with other sources, the seeding accounted for 13.7–16.4% of the species but a comparatively large 39.6–41.8% of the maximum cover. Thus, seeding does appear to contribute disproportionately to coverage. The WIS score for the species we found in our sampling plots that were in the seeding mix was 3.8, which is much closer to the WIS score for the lowland seed mix (4.0) than the upland mix (2.0; Table 1). Thus, the lowland mix appears to have had more of an impact that the upland, suggesting that the former is a better one to use in wetland restoration in our area. Relatively low representation of species from introduced propagules has been seen in other restored wetlands (*e.g.*, Fink and Mitsch 2007). By contrast, Mitsch *et al*. (2012) found that nine of 13 native species planted were still present in a restored wetland in central Ohio after 15 years. Future work is needed to show if seeding at this site requires a longer time period to accurately assess its total impact.

The third potential source of propagules was from Pond 1, and 10 species show evidence of propagating from this source (Fig. 5). Of those, *Cyperus strigosus* and *Hibiscus moscheutos* were also seeded, but Pond 1 could also have served as a propagule source. Thus, 11–14% of the species we found show strong evidence of spreading from Pond 1. If Pond 1 was an important propagule source for *C. strigosus* and *H. moscheutos*, it may have accounted for as much as 11.4% of cover; otherwise, it only accounted for 9.1%. These numbers could be conservative if there were species that spread quickly from Pond 1 and fully occupied the site in the four years between pond construction and this study. This shows that remnant wetland fragments, if they are available, can be important in overall restoration of a site.

The GAM surface contour fit of the second NMDS axis with relative elevation, as well as the correlation between this axis and relative elevation, confirm our hypothesis that elevational topography is a major driver of plant composition. The GAM surface contour fit for the distance from the center of Pond 1 on the first NMDS axis and the correlation between this axis and distance from the center of Pond 1 also support our hypothesis that distance from Pond 1 is an important factor in plant composition; however, the change in plant composition along this axis may additionally be driven by other factors not measured in this study. Plot meanCC was correlated with the first NMDS axis, which suggests a higher floristic quality toward the Pond 1 end of the axis. As Pond 1 is older than the other ponds, it may have had more time to become a higher quality wetland than the other ponds and serve as a source of nonintervention plants. Alternatively, these patterns may simply be a reflection of better establishment sites for species along the gradient from Pond 1 to Pond 6. There are differences in plot median relative elevations among the six ponds (data not shown); however, the distributions of relative elevations show substantial overlap among all of the ponds, as can be seen in Table 2.

Relative water depths in Pond 6 varied from 11.4 cm below to 26.5 cm above the mean water level over the 2-year measured period, while vegetation sampling plots ranged in elevation from –48 to 51 cm above this same mean water level (Fig. 2, Table 2). Thus, there are some sampling plots that were always inundated, while others never were. For 50% of the measured period, water levels ranged between –2.7 and 4.6 cm, but $<$ 10% of the sampling plots fell into this range. We included a range of sampling plots with quite different hydroperiods, due to their differing elevation, and thus expected to see a strong effect of elevation, which indeed was strongly associated with the second NMDS axis.

Given that fewer plants can tolerate constantly inundated conditions, it was no surprise that cover increased with elevation. The correlation with WIS score also was unsurprising, since that reflects inundation levels, as well. The species that favor the lowest elevations are all classified as obligate wetland species. The species that favor the highest elevations, however, show a broader range, from FACW to UPL, as might be expected from plots with different amounts of inundation and water availability. The positive correlation between elevation and nonnative cover and fraction of nonnative species has been seen in a study of restored wetlands in Iowa (Seabloom and van der Valk 2003), and it suggests there is a larger pool of potential nonnative species for upland areas. However, one of the invaders at our site is *Lythrum salicaria*, a highly invasive obligate wetland plant, which may change this correlation with time if not removed. Mitsch *et al*. (2012) found only 7 nonnative species 15 years after wetland restoration, *vs*. the 14 found in this study after four years. This could be caused by a number of factors, including differences in wetland type, sampling techniques, and site history.

Conclusion. Our data show mixed success in the restoration of this site. Although the low VIBI score places it in the most disturbed KY-WRAM category, comparison of our site with other restored wetlands in the region suggests we achieved similar or slightly better results. To our knowledge, this is the first study that has attempted to quantify propagule sources and their importance in restoration. We found that seedbank and/or offsite sources were the most important in terms of both plant diversity and cover. While seeding was not a large contributor to diversity, it was quite important in terms of cover, and the lowland seed mix appears to have had more of an impact than the upland mix. The onsite source of propagules (Pond 1) contributed on the order 10–15% for both overall diversity and cover, but this estimate is probably conservative. In addition, the onsite propagule source was an important determinant of plant patterns at this site, as distance from the center of Pond 1 was correlated with one of the NMDS axes. Elevation relative to mean water level was the other important factor determining plant patterns.

Literature Cited

Andreas, B. K., J. J. Mack, and J. S. McCormac. 2004. Floristic Quality Assessment Index (FQAI) for vascular plants and mosses for the State of Ohio. Ohio Environmental Protection Agency, Division of Surface Water, Wetland Ecology Group, Columbus, OH, USA. 219 pp.

Balcombe, C. K., J. T. Anderson, R. H. Fortney, J. S. Rentch, W. N. Grafton, and W. S. Kordek. 2005. A comparison of plant communities in mitigation and reference wetlands in the mid-Appalachians. Wetlands 25: 130–142.

Biebighauser, T. R. 2007. Wetland Drainage, Restoration, and Repair. University of Kentucky Press, Lexington, KY, USA. 252 pp.

Boyce, R. L., R. D. Durtsche, and S. L. Fugal. 2012. Impact of the invasive shrub *Lonicera maackii* on stand transpiration and ecosystem hydrology in a wetland forest. Biological Invasions 14: 671–680.

Braun, E. L. 1916. The physiographic ecology of the Cincinnati region. Bulletin of the Ohio Biological Survey 2: 113–211.

Bryant, W. S. 1987. Structure and composition of the old-growth forests of Hamilton County, Ohio and environs, pp. 317–324. *In* R. L. Hay, F. W. Woods, and H. DeSelm, eds., Proceedings of the 6th Central Hardwoods Forest Conference. University of Tennessee, Knoxville, TN, USA. 618 pp.

Bryant, W. and M. Held. 2004. Forest vegetation in Hamilton County, Ohio: A cluster analysis and ordination study, pp. 312–321. *In* D. A. Yaussy, D. M. Hix, R. P. Long, and P. C. Goebel, eds., Proceedings of the 14th Central Hardwoods Forest Conference. General Technical Report NE-316. USDA Forest Service, Northeastern Research Station, Newtown Square, PA, USA. 539 pp.

Dahl, T. E. 2011. Status and trends of wetlands in the conterminous United States 2004 to 2009. Retrieved May 13, 2019, from U.S. Department of the Interior, U.S. Fish and Wildlife Service, Fisheries and Habitat Conservation, Washington, DC <https://www.fws.gov/wetlands/status-and-trends/Status-and-Trends-2004-2009.html>.

Fink, D. F, and W. J. Mitsch. 2007. Hydrology and nutrient biogeochemistry in a created river diversion oxbow wetland. Ecological Engineering 30: 93–102.

Gianopulos, K. 2014. Coefficient of conservatism database development for wetland plants occurring in the Southeast United States. North Carolina Dept. of Environment & Natural Resources, Division of Water Resources: Wetlands Branch. Report to the EPA, Region 4. Retrieved September 18, 2019, from <http://www.ncwetlands.org/wp-content/uploads/Southeast-Wetland-Plant-CofC-Database-Development-Report-FINAL-2014.pdf>.

Henry, H. A. L., S. D. Murphy, M. L. McFarlane, H. Barna, K. Dunning, A. Hood, and J. C. Crosthwaite. 2018. Evaluating outcomes of restoration ecology projects on limited budgets: Assessment of variation in sampling intensity and sampling frequency for four habitat types. Restoration Ecology 27: 457–465.

Jones, R. L. 2005. Plant Life of Kentucky. University Press of Kentucky, Lexington, KY. 856 pp.

Kentucky Division of Water. 2019. Kentucky Wetlands Rapid Assessment Method— Kentucky Energy and Environment Cabinet. Retrieved May 13, 2019 from Kentucky Energy and Environment Cabinet <http://eec.ky.gov/Environmental-Protection/Water/Monitor/Pages/KYWRAM.aspx>.

LINK-GIS. 2016. LINK-GIS Map Viewer. Retrieved July 5, 2016 from <http://linkgis.org/mapviewer/index.html>.

Matthews, J. W. and G. Spyreas. 2010. Convergence and divergence in plant community trajectories as a framework for monitoring wetland restoration progress. Journal of Applied Ecology 47: 1128–1136.

Meyer, C. K., M. R. Whiles, and S. G. Baer. 2010. Plant community recovery following restoration in temporally variable riparian wetlands. Restoration Ecology 18: 52–64.

Miller, S. J. and D. H. Wardrop. 2006. Adapting the floristic quality assessment index to indicate anthropogenic disturbance in central Pennsylvania wetlands. Ecological Indicators 6: 313–326.

Mitsch, W. J. and J. W. Day, Jr. 2006. Restoration of wetlands in the Mississippi–Ohio–Missouri (MOM) River Basin: Experience and needed research. Ecological Engineering 26: 55–69.

Mitsch, W. J., L. Zhang, K. C. Stefanik, A. M. Nahlik, C. J. Anderson, B. Bernal, M. Hernandez, and K. Song. 2012. Creating wetlands: Primary succession, water quality changes, and self-design over 15 years. BioScience 62: 237–250.

Pfister, R. D., B. L. Kovalchik, S. F. Arno, and R. C. Presby. 1977. Forest Habitat Types of Montana. U.S. Department of Agriculture, Forest Service, Intermountain Forest & Range Experiment Station, Ogden, UT, USA. 174 pp.

R Core Team. 2019. R: A language and environment for statistical computing. Retrieved May 13, 2019 from R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Reeder, B. C. 2011. Assessing constructed wetland functional success using diel changes in dissolved oxygen, pH, and temperature in submerged, emergent, and open-water habitats in the Beaver Creek Wetlands Complex, Kentucky (USA). Ecological Engineering 37: 1772–1778.

Seabloom, E. W. and A. G. van der Valk. 2003. Plant diversity, composition, and invasion of restored and natural prairie pothole wetlands: Implications for restoration. Wetlands 23: 1–12.

Smith, N. N. 2016. A vegetation-based index of biotic integrity for wetlands of Kentucky. Masters thesis. Eastern Kentucky University, Richmond, KY. Retrieved May 13, 2019 from <https://encompass.eku.edu/cgi/viewcontent.cgi?article=1425&context=etd>.

Spieles, D. J. 2005. Vegetation development in created, restored, and enhanced mitigation wetland banks of the United States. Wetlands 25: 51–63.

Swink, F. and G. Wilhelm. 1994. Plants of the Chicago Region, 4th ed. Indiana Academy of Science, Indianapolis, IN, USA. 921 pp.

Tu, M., B. Rice, and J. Randall. 2002. Element stewardship abstract for *Lespedeza cuneata*. Retrieved May 13, 2019 from Center for Invasive Species and Ecosystem Health, University of Georgia, Athens, GA, USA. <https://www.invasive.org/weedcd/pdfs/tncweeds/lespcun.pdf>.

U.S. Army Corps of Engineers. 1987. Wetland delineation manual. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, USA. Technical Report Y-87-1. Retrieved May 13, 2019 from <https://www.lrh.usace.army.mil/Portals/38/docs/USACE%2087%20Wetland%20Delineation%20Manual.pdf>.

[US EPA] U.S. Environmental Protection Agency. 2006. Elements of a state water monitoring and assessment program for wetlands. Office of Wetlands, Oceans, and Watersheds, U.S. Environmental Protection Agency, Washington, DC. Retrieved May 13, 2019 from <https://www.cramwetlands.org/sites/default/files/Wetland_Elements_Final.pdf>.

[USDA NRCS] U.S. Department of Agriculture-Natural Resources Conservation Service. 2019. Welcome to the PLANTS Database. Retrieved May 13, 2019 from <https://plants.sc.egov.usda.gov/java/>.

Vileisis, A. 1997. Discovering the Unknown Landscape: A History of America's Wetlands. Island Press, Washington, DC. 433 pp.

Wentworth, T. R., G.P. Johnson, and R. L. Kologiski. 1988. Designation of wetlands by weighted averages of vegetation data: A preliminary evaluation. Journal of the American Water Resources Association 24: 389–396.

Zar, J. H. 2010. Biostatistical Analysis, 5th ed. Prentice Hall, Upper Saddle River, NJ, USA. 944 pp.