

Development of Missouri Reference Wetlands

October 2017

By

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For Missouri Department of Natural Resources

**Prepared in fulfillment of
G14-WET-01 and KUCR STE72795**

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Summary

The objective of this project was to identify candidate reference wetlands in Missouri using existing landscape-level data, and begin development of a quantitative, scientifically defensible method to determine candidate reference site conditions in Missouri wetlands. The study was restricted to non-forested, palustrine wetlands greater than 10 acres in size, within the Missouri River and major tributary floodplains situated in the Western Corn Belt Plains and Central Irregular Plains ecoregions. A GIS-based assessment of land use within a 250m buffer around each potential wetland was applied to select for field assessment two groups of wetlands – a group with high agricultural influence and a more natural group with low agricultural impact that could serve as reference wetlands. A third group of hand-picked wetlands considered by best professional judgment to be of good quality was also field assessed. Twenty-six wetlands were field-assessed once each during this project. Resulting water chemistry and biological data were statistically analyzed to determine if the GIS-based assessment adequately differentiated the more natural wetlands from those under heavy agricultural influence. We expected the more natural grouping to have higher macroinvertebrate diversity, greater wetland plant diversity and more obligate species, and better water quality than the agricultural sites. However, the only meaningful relationship we found was that agricultural sites had fewer obligate wetland plant species than either natural or hand-picked wetlands, which could be related to the isolation of the agricultural wetlands. Refining land use metric application and increasing the number of sampling events might increase the power of these methods to discern reference wetland sites which in turn would provide data to develop scientifically defensible water quality standards for wetlands.

Background

Wetlands provide key habitats for amphibians, fish, waterfowl, and aquatic invertebrates, while also providing essential ecosystem services for human uses. Reduction of floodplain connectivity, channelization and damming, wetland draining, and human development have dramatically reduced the amount of wetland habitat available in Missouri, leading to degraded conditions and loss of aquatic biodiversity. Remaining wetlands in the state vary in functioning and degree of human impact; however, designated standards for water quality and habitat conditions have not been set for Missouri wetlands.

The Environmental Protection Agency (EPA) has instituted a national effort to encourage and support the development of state wetland programs, and has identified four core elements (the Core Elements Framework) that comprise and strengthen effective state and tribal wetlands programs (EPA 2009). One of these core elements is the development of scientifically defensible water quality standards for wetlands. Although Missouri's water quality standards define and address wetlands in general, Missouri currently does not have water quality

standards for wetlands, including wetland-specific designated uses, criteria to protect those uses, and a dataset of classified wetlands to which these uses and criteria would apply. A lack of water quality and other supporting data necessary to classify and identify wetland uses currently precludes development of wetlands-specific water quality standards at this time.

As part of its Water Quality Standards triennial review process, the MDNR will consider establishment of wetland water quality standards. The goal of this grant project is to establish a set of reference wetlands in Missouri, with potential emphasis on riparian wetlands in floodplains of the Missouri River and its tributaries. Reference wetlands identified in these systems may be used as a foundation upon which to base wetland water quality standards (appropriate designated uses, numeric criteria to protect those uses, and antidegradation) and establish an Index of Biotic Integrity for wetlands in Missouri.

LiDAR elevation dataset preparation and processing

The study area includes the Missouri River floodplain along with major tributary floodplains flowing in from the north and inside the Missouri state boundary (Figure 1). To help identify the target wetland population, a study area mask for these floodplains was developed using GIS. To ensure proper watershed size (flow accumulation) determination for stream network delineation, LiDAR bare earth elevation data were obtained for a bigger region that included parts of Iowa (<https://programs.iowadnr.gov/nrgislibx/>), Nebraska (<https://dnr.nebraska.gov/data/elevation-data>), and Kansas (<https://www.kansasgis.org/>), in addition to Missouri (USDA Natural Resources Conservation Service, personal communication and custom data transfer). The state-specific LiDAR data were mosaicked and projected to a common 10 m grid in the UTM15N projection. These state-specific elevation datasets were inspected for holes; 1179 small areas of missing data (1034 in NE, 145 in MO) were identified and filled in using nearby data interpolated across the missing areas using triangulated irregular networks. Following hole-filling, the state-level datasets were mosaicked to create a single elevation coverage for the study area. Four additional holes along state collection boundaries were identified and filled in, and the data were then reprojected to a 30 m grid to facilitate large area processing.

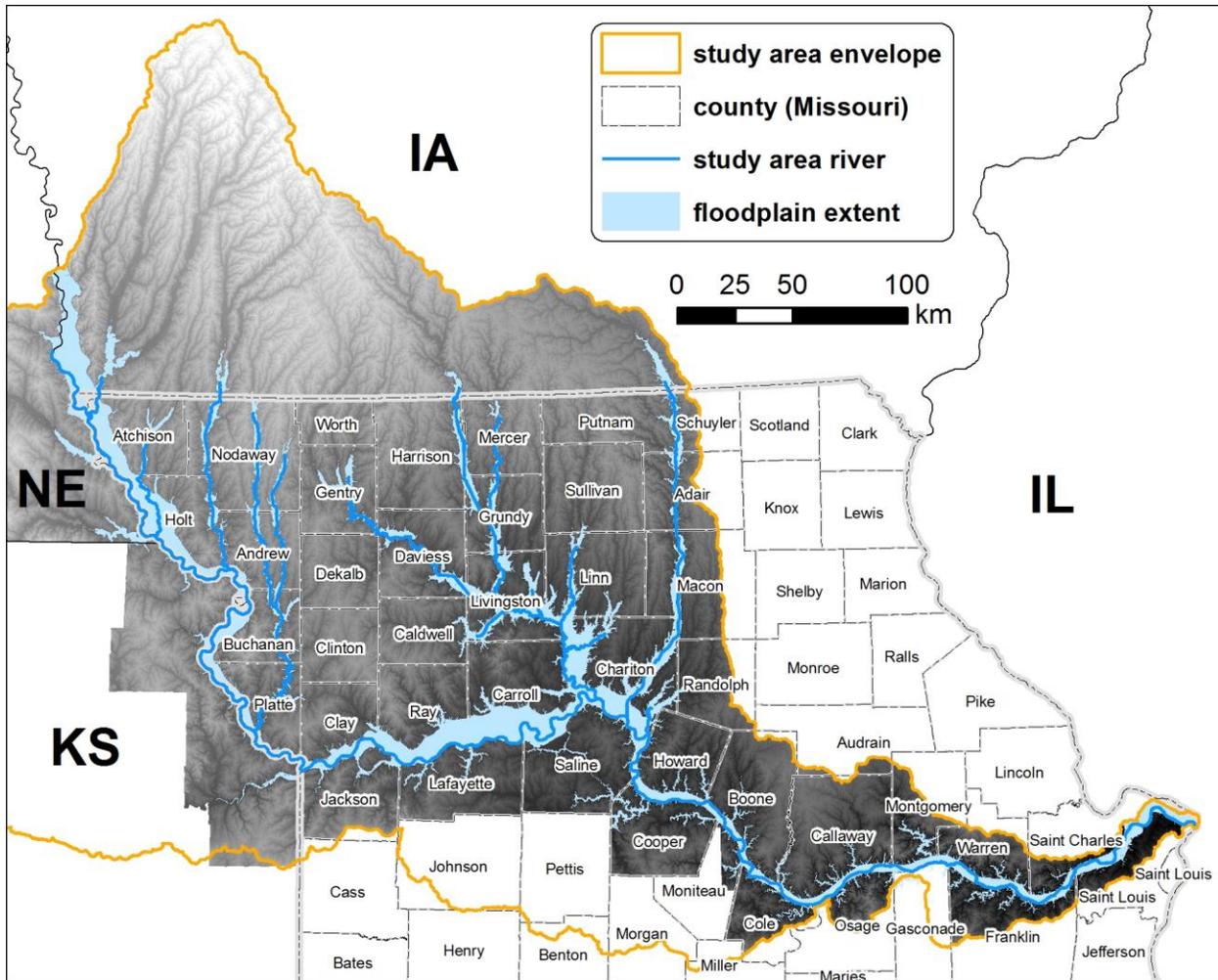


Figure 1. Study area includes the floodplains of the Missouri River and its primary tributaries (minimum catchment = 640 km²) flowing in from the north. The LiDAR elevation dataset assembled for the project is shown in the background.

Two data gaps along the Missouri River floodplain (primarily in Gasconade and Osage counties) were filled in using data downloaded from the USGS National Elevation Dataset (NED). Next, the dataset was examined for hydro-enforcement needs, whereby obstructed flow passages (typically occurring at bridges or culverts) are breached in the elevation dataset to facilitate accurate hydrologic processing. To identify possible locations warranting hydro-enforcement, 9611 depressions were identified throughout the study area that had a maximum depth of at least 2 m. Upon inspection, 80 were found to occur within the study area floodplains using a preliminary floodplain extent map. These obstructions were punctured (breached) in the elevation dataset using standard GIS processing techniques.

With the LiDAR elevation dataset prepared as described above, it was then subjected to basic hydrologic processing (Jenson and Domingue 1988) to obtain the data layers needed for floodplain mapping (Task 1). First, all depressions were filled using the Arc Hydro Tools extension for ArcGIS. Next, pixel-level D8 flow direction was determined for the depressionless elevation dataset. Lastly, the flow direction raster was used to determine pixel-level flow accumulation (catchment size) values. Through inspection and trial and error, a minimum catchment threshold of 640 km² was determined to provide a reasonable representation of the desired stream network corresponding to the major drainages across the study area, while also capturing the majority of the hand-picked candidate wetland sites on the list provided by MDNR.

For the final data preparation step in advance of floodplain mapping, the stream network was pared to exclude reaches outside of the study area (i.e. stream segments outside of Missouri or south of the Missouri River were deleted). The remaining stream network was then processed using the FLDPLN (“Floodplain”) model (Kastens 2008, Williams et al. 2013). *Depth to flood* (DTF) is the key parameter for FLDPLN, which estimates inundation extent at various river stage values using basic hydrologic flow principles applied to a targeted stream reach. The larger the maximum DTF value, the greater the inundated area. DTF is analogous to a river stage value using stream pixel elevations as location-specific datum values. Through inspection and previous research in the study area, we determined that a maximum DTF value of 16 m well captured the Missouri River floodplain (valley floor) extent, whereas a maximum DTF value of 12 m was appropriate for capturing all of the tributary floodplain extents. The total merged floodplain extent using these DTF values is shown in Figure 1. This floodplain mask was subsequently used to identify floodplain wetlands, which were the original target of this study.

Wetland Target Population Development (Task 2)

The National Wetland Inventory (NWI) polygon dataset for Missouri was downloaded from the USFW data portal (<https://www.fws.gov/wetlands/>). Clipping the NWI to the study area envelope, the initial wetland population consisted of 366,471 features. Several additional restrictions were imposed to determine the target population, which was defined to be non-forested, palustrine wetlands at least 5 acres in size and which occurred in the floodplains of the study area stream network. Application of these criteria resulted in a target population consisting of 3485 NWI features. Twenty-six preferred (hand-picked) sampling sites were provided by MDNR, of which six were not represented in the reduced NWI dataset. These features were added (four from NWI that did not satisfy all the selection criteria, plus two manually delineated using aerial imagery and LiDAR), bringing the total to 3488 wetland features.

General incongruence between wetland polygons and corresponding features visible in LiDAR prohibited the delineation of meaningful, wetland-specific catchments to use for wetland landscape characterization (Figure 2). Consequently, a traditional fixed-width buffering approach (250 m in this case) was used instead to identify wetland contributing area ([Task 3](#)).

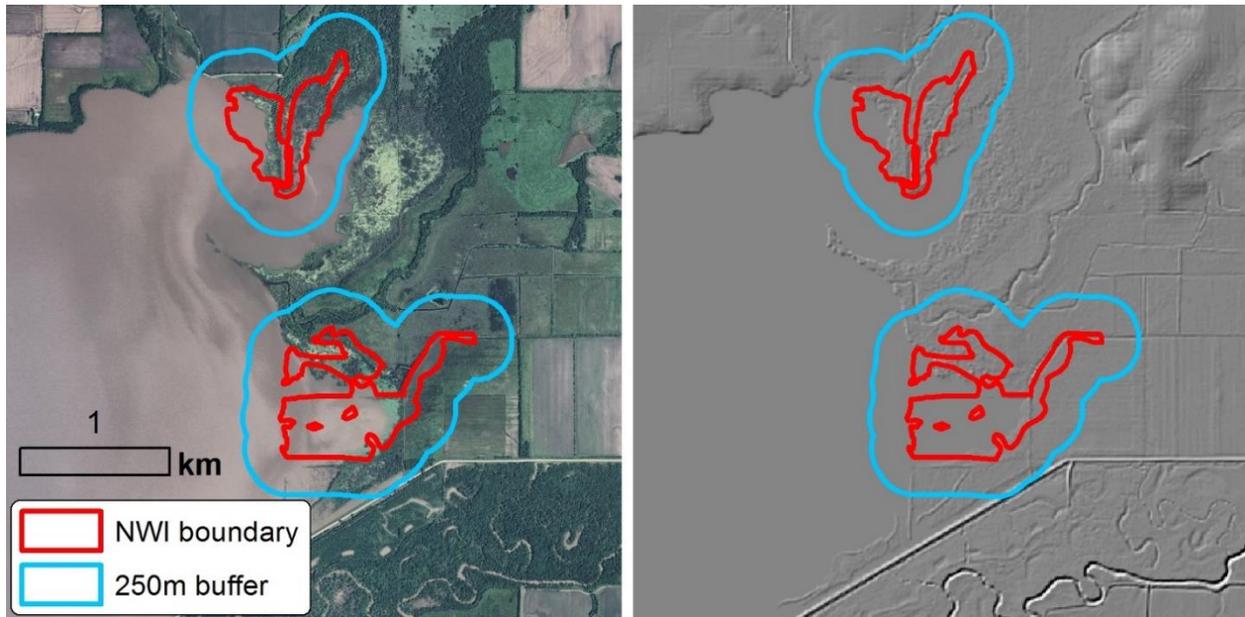


Figure 2. National Wetland Inventory (NWI) boundaries and 250m buffers for study sites 2883 (north) and 2810 (south) (polygons 5002 and 2729, respectively, in the associated shapefile). The background on the left is NAIP 2010 imagery; the background on the right is LiDAR shaded relief. General incongruence between wetland polygons and corresponding features visible in LiDAR prohibited the delineation of wetland-specific catchments to use for wetland landscape characterization.

Land cover data from the USGS National Land Cover Dataset 2011 (NLCD; Homer et al. 2015) were extracted for each buffered wetland area. These land cover data were then transformed to values reflective of their nutrient loss potential (EPA 2002) and degree of landscape disturbance (Brown and Vivas 2005).

Using information from Table 1 in EPA 2002, estimated nitrogen loss rates (NLR) and phosphorous loss rates were (PLR) were assigned to each land cover class found in the study area's NLCD data (Table 1, [Task 4](#)). Landscape Disturbance Index (LDI) coefficients were similarly assigned to NLCD classes using information found in Table 2 of Brown and Vivas 2005 (Table 1). Zonal average NLR, PLR, and LDI values were computed for each buffered wetland polygon to obtain representative values for each wetland (Figure 3).

Table 1. Land cover classes in the study area's USGS National Land Cover Dataset 2011 (NLCD), with potential nitrogen (N_coef) or phosphorus (P_coef) loss rates (kg/ha/yr) (from Table 1 EPA 2002) and landscape development intensity index coefficients (LDI_coef, from Table 2 Brown and Vivas). Where two LDI classes are listed, an average value was used.

NLCD_code	NLCD_class	N_coef	P_coef	NLI_class	LDI_coef	LDI_class1	LDI_class2
11	Open Water	0.00	0.0000	Not Applicable	1.00	Natural open water	
12	Perennial Ice/Snow	0.00	0.0000	Not Applicable	1.00	Natural open water	
21	Developed, Open Space	0.55	0.0190	Mixed	4.37	Recreational/open space - low-intensity (1.83)	Single family residential - low-density (6.9)
22	Developed, Low Intensity	0.55	0.0190	Mixed	7.47	Single family residential - medium density	
23	Developed, Medium Intensity	0.79	0.0300	Mostly urban	7.78	Single family residential - high density (7.55)	Low-intensity commercial (8)
24	Developed High Intensity	0.79	0.0300	Mostly urban	9.18	High-intensity commercial	
31	Barren Land (Rock/Sand/Clay)	0.00	0.0000	Not Applicable	1.00	Natural system	
41	Deciduous Forest	0.44	0.0085	Natural vegetation	1.00	Natural system	
42	Evergreen Forest	0.44	0.0085	Natural vegetation	1.00	Natural system	
43	Mixed Forest	0.44	0.0085	Natural vegetation	1.00	Natural system	
51	Dwarf Scrub	0.44	0.0085	Natural vegetation	1.00	Natural system	
52	Shrub/Scrub	0.44	0.0085	Natural vegetation	1.00	Natural system	
71	Grassland/Herbaceous	0.44	0.0085	Natural vegetation	3.41	Improved pasture - low-intensity (with livestock)	
81	Pasture/Hay	0.45	0.0180	Mostly natural vegetation	3.74	Improved pasture - high-intensity (with livestock)	
82	Cultivated Crops	0.98	0.0310	Agricultural	4.54	Row crops	
90	Woody Wetlands	0.44	0.0085	Natural vegetation	1.00	Natural system	
95	Emergent Herbaceous Wetlands	0.44	0.0085	Natural vegetation	1.00	Natural system	

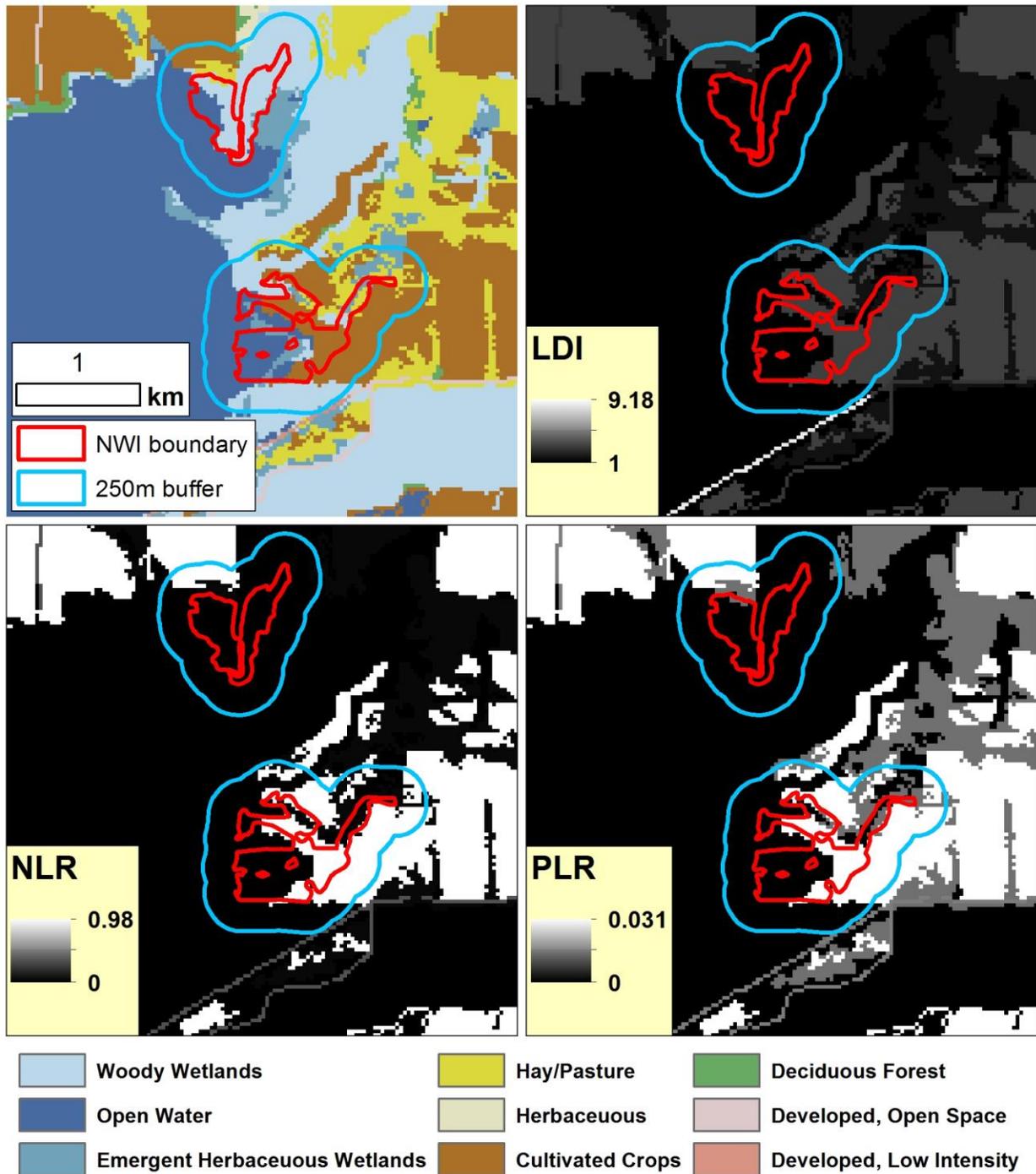


Figure 3. Example of translation of USGS National Land Cover Dataset 2011 (NLCD) classes (upper left image) to landscape development intensity index (LDI), nitrogen loss rate (NLR) and phosphorus loss rate (PLR) values for study sites 2883 (north) and 2810 (south). Legend at bottom describes NLCD classes. For each index, lower values represent a more natural landscape.

A list of wetlands with LDI, NLR, and PLR values was exported into MSEXcel and MSAccess to further examine them for sampling (Appendix A). Wetlands were ranked by each of the three extracted statistics, taking into account ties (i.e., polygons that tied received the same rank, and then the next one down the list received one lower rank, etc.). We first examined a composite rank calculated from the sum of the three rank order values. This treats all three values with equal importance and makes no account for any specific value jumps. During this examination we realized that N and P loss was mutually exclusive with the LDI for urban sites. In other words, urban sites which we assume to have highly impacted wetlands, have low nitrogen and phosphorus loss values. As expected the highly impacted agricultural sites have high NLR and PLR values. Thus, after discussion with the MDNR project officer, we decided to exclude urban sites from this study, and focus on agricultural sites (highest indices after urban excluded) and natural landscape (lowest indices) sites.

We narrowed this list to those NWI polygons > 10 acres, which resulted in 1512 polygons representing palustrine wetlands from which we selected wetlands to sample ([Task 5](#)). Indices ranges within this 1512 polygon set were: LDI 1 (most natural) to 7.19; NLR 0.139 (least nitrogen loss) to 0.98; and PLR 0.004 (least phosphorus loss) to 0.031.

Sample site selection

Natural sites

To narrow down the polygon set to two groups of approximately 10 wetlands on opposite ends of the land use spectrum (natural versus agricultural), the polygons were sorted by ascending LDI value and visually examined in Google Earth Pro ([Task 8](#)). Polygons that were long and linear (ditches), within rivers, most likely dry (examined over a period of years), and forested within the polygon were rejected. To select the most natural, least agriculturally influenced sites, we examined the list of polygons in order of ascending LDI to a maximum of LDI of 3.00 and field verified that the polygons were wetlands and accessible. We sampled 10 polygons in this group.

Agricultural sites

Selection of the agriculturally-influenced polygons required more screening by site indices. Since LDI >7 represents medium to high urban landscape (as defined by the LDI coding, and confirmed by mapping), we screened out the two polygons with LDI > 7. Next, we examined polygons with LDI 3 to 7, which represents agricultural landscape. All 14 polygons with LDI 5 to 7 were located in urban areas, so we limited the list to polygons with LDI 3 to 5. Within this list we examined polygons with the highest NLR (nitrogen loss rate) and PLR (phosphorus loss rate) values. In the entire set of 1512 polygons, the approximately 300 polygons with highest NLR and PLR had LDI values in the range of 4 to 5, confirming that high NLR and PLR reflects

agricultural landscapes. As NLR and PLR are highly correlated (Figure 4), we focused office and field verification on polygons with the highest NLR (0.6 to 0.98) and LDI 3 to < 5. It was very fruitful to focus on those polygons marked as wetlands in a gazetteer. We sampled 9 polygons in this group.

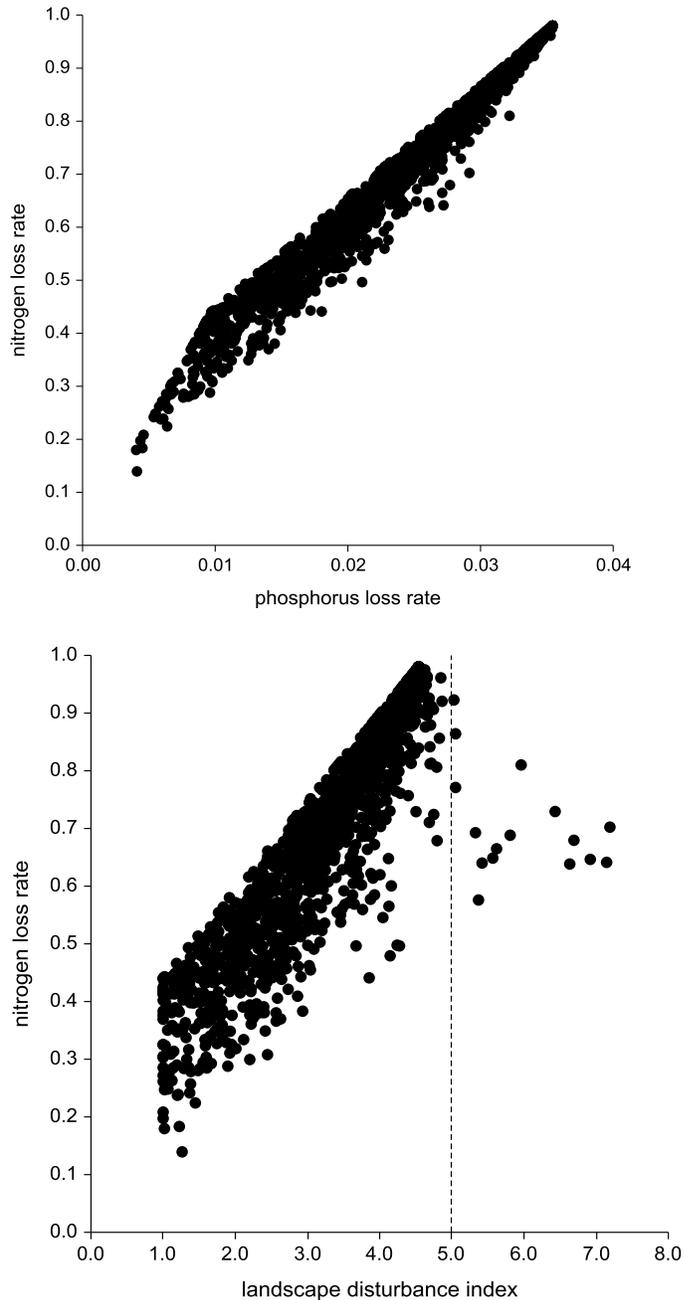


Figure 4. Nitrogen loss rate (NLR) versus phosphorus loss rate and landscape disturbance index (LDI). LDI above 5 (dotted line) represents urban land use, at which point the upwards trend with increased NLR falls apart.

We evaluated 425 polygons in the office, and marked 135 of these as potential sampling sites to visit in the field. We pursued land owner permission for 51 of these polygons, and were given permission to sample 35 polygons. Final sampling was based on accessibility and water present at the time of sampling. We sampled 10 natural sites and 9 agricultural sites (Figure 5).

Hand-picked sites

We initially proposed to sample randomly-selected sites that fell along the landscape and nutrient gradient midway between the natural and agricultural site, but after discussion with MDNR project officer instead sampled 7 hand-picked wetlands chosen by best professional judgment to be of high quality (most natural). Six of these did not fall within polygons provided in the original set of 1512 polygons, so we retroactively calculated the landscape and nutrient indices.

Location verification and reconciliation

Post-field work, sample points were examined in Google Earth Pro and GIS to confirm they were in the intended polygons. Two sample locations were not in the intended polygons so the true polygon codes were assigned to the collected data. Three hand-picked sites did not fall in any polygons, so polygons were hand digitized and indices determined. The mismatch of intended sites with NWI polygons, or lack of polygons, may be the result of GPS error (3 – 6 m) when locating the sites, or NWI boundaries based on old imagery.

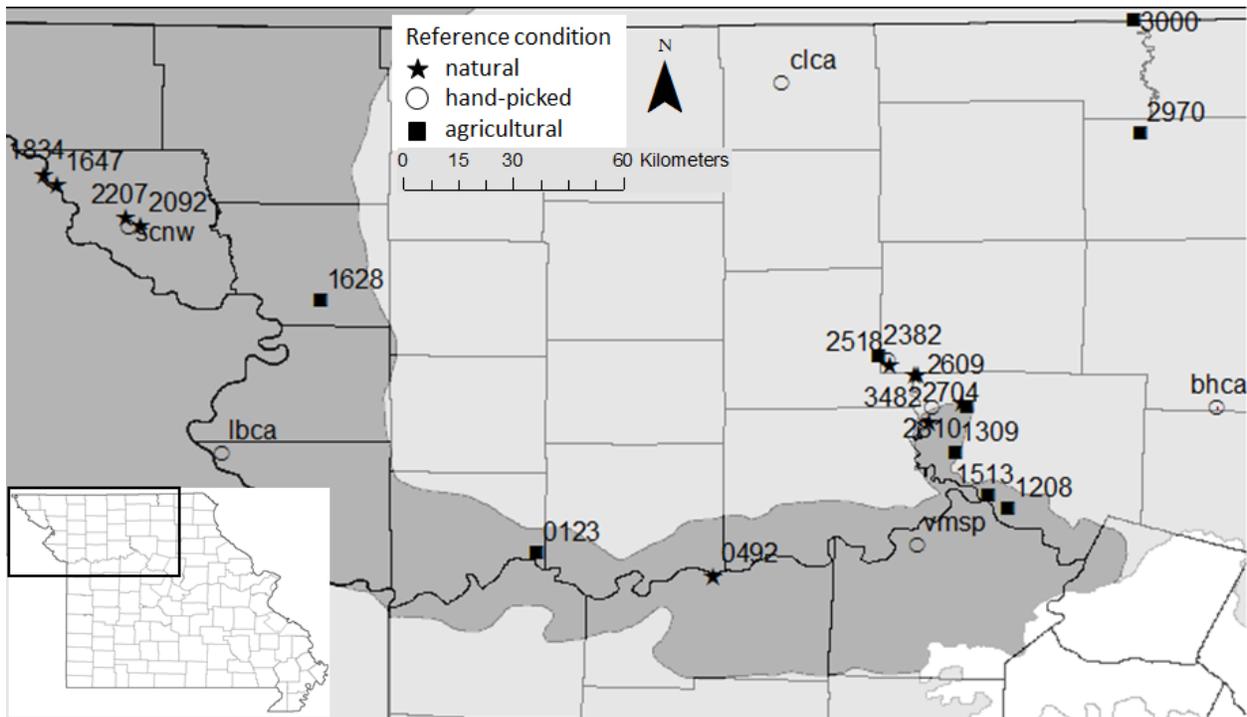


Figure 5. Sampling sites in northwest Missouri (inset), with Omernik Level 3 ecoregions shaded as dark gray for Western Corn Belt Plains and light gray for Central Irregular Plains (see <https://www.epa.gov/eco-research/ecoregions>).

Field methods (Tasks 10 and 11)

Field methods are detailed in the Quality Assurance Project Plan, and summarized here. Field work consisted of walking or canoeing into each wetland to collect water and macroinvertebrates samples, measure *in situ* water chemistry, and evaluate vegetation. Water samples and measurements were made before the site was disturbed by the other activities.

Water

At two locations in each wetland sufficient water was collected to fill containers that were shipped on ice to the MDNR lab for analyses of metals, nutrients, and other elements (Table 2). One site was reduced to a small pool from which we collected only 1 water sample. Five locations were sampled twice for laboratory QAQC, for a total of 56 samples from 26 wetlands. At the two water sample locations, plus a third, the following *in situ* water chemistry parameters were measured with a Horiba U-52 water quality checker: temperature, dissolved oxygen, conductivity, turbidity, oxidation-reduction potential (ORP), total dissolved solids (TDS) and salinity.

Table 2. MDNR chemical analysis methods for field-collected water samples.

Container	Filter	Acidify	Parameter	Lab method	Unit
500 ml	yes	HNO ₃	Dissolved Calcium	EPA 200.7	mg/L
			Dissolved Magnesium	EPA 200.7	mg/L
			Dissolved Cadmium	EPA 200.8	µg/L
			Dissolved Copper	EPA 200.8	µg/L
			Dissolved Lead	EPA 200.8	µg/L
			Dissolved Zinc	EPA 200.8	µg/L
			Hardness (as CaCO ₃ -TR-N)	SM 2340-B	mg/L
1000 ml	no	H ₂ SO ₄	Total Nitrogen (TN)	USGS I-2650-03 modified	mg/L
			Total Phosphorus (TP)	USGS I-2650-03 modified	mg/L
1000 ml	no	no	Non Filterable Residue (NFR) (Total Suspended Solids TSS)	SM 2540-D	mg/L
			Total Dissolved Solids (TDS)	SM 2540C	mg/L
			Chloride (Cl)	SM 4500-Cl-E	mg/L
2 VOA vials	no	H ₃ PO ₄	Total Organic Carbon (TOC)	SM 5310C	mg/L

Macroinvertebrates

Macroinvertebrate sampling was conducted within each habitat type in proportion to the amount of that habitat in the wetland, for a total of 3 minutes of sampling (Huggins and Moffet 1988). For each sample within a habitat, a kick and sweep method with a 500-micron D-frame aquatic net was used to capture invertebrates in the benthos substrate by disturbing the surface of the benthos for 30 seconds while sweeping the net through the water column directly above the turbulence. Samples from each wetland were composited and preserved with 10% buffered formalin with rose Bengal. At the KBS labs, samples were rinsed of field fixative and sorted to a 200 organism count in a gridded Canton tray, using USEPA EMAP methods (USEPA 1995, USEPA 2004) as explained in the Standard Operating Procedure (SOP) of the CPCB at the KBS (Blackwood 2007). Specimens were identified to the lowest taxonomic level practical, which is genus level for most taxonomic groups when possible (Blackwood 2007, MDNR-ESP-209). Data were entered into an MSAccess database and a number of community metrics calculated using EcoMeas (version 1.6) a software program that calculates varies diversity and community metrics.

Vegetation

At each wetland, a floristic quality assessment (FQA) for each non-woody, palustrine community at least 1.2 ha (3 ac) in area was conducted (Kriz et al. 2007). A master species checklist for palustrine communities was used to record each native and naturalized species observed within each plant community. Plants that could not be identified in the field were collected, pressed, and taken to the R.L. McGregor Herbarium, University of Kansas (KANU) for identification. Vouchers were deposited at KANU. Vegetation canopy cover within each primary plant community was estimated within a 10 m² circular plot. Presence/absence data from the FQA were entered into MSExcel and uploaded to an online FQA calculator loaded with Missouri-specific coefficients of conservatism. The following site metrics were calculated: total and native species richness, percent non-native species, mean conservatism (all species), mean conservatism (native species only), total floristic quality index (FQI), native FQI, mean wetness, and native mean wetness.

Data analyses (Task 12)

The goal of this study was to examine the appropriateness of a GIS-based land use assessment method to identify wetlands that meet reference criteria. We compared biota and water chemistry of wetlands located in landscapes with low human influence to biota and water chemistry of wetlands 1) in agricultural landscapes and 2) designated as reference by the USEPA Region 7 Technical Assistance Group (RTAG).

Land use indices

Wetlands were plotted to examine where hand-picked sites fell on the continuum of low to high land use and nutrient loss rates (Figure 6). They fell between the two extremes of natural verses agricultural sites, with some overlap (Table 3).

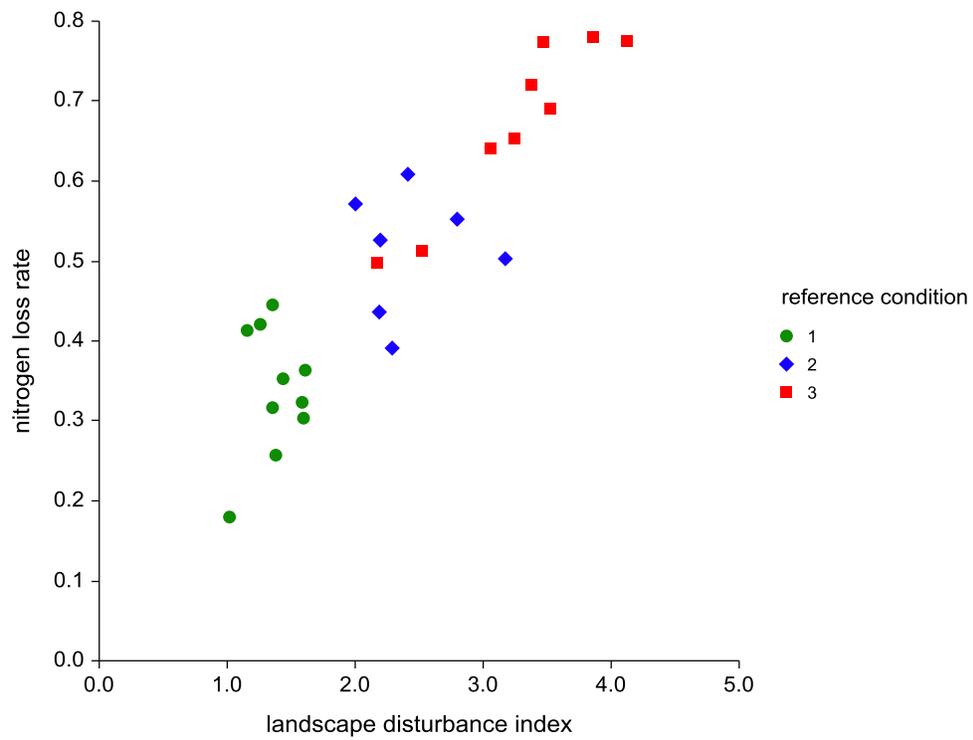
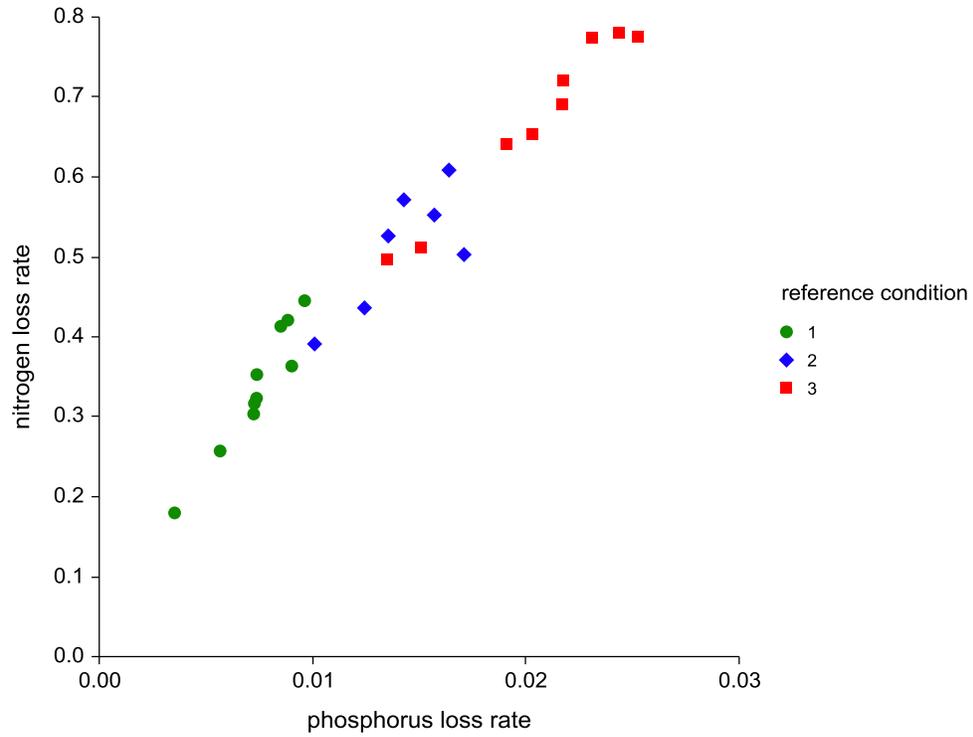


Figure 6. Scatter plots for nitrogen loss rate versus phosphorus loss rate or landscape disturbance index. Reference conditions: 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands.

Table 3. Descriptive statistics for land use indices, by land use codes: all (n =26), natural (low human land use, n = 10), hand-picked (n = 7), and agricultural (n = 9) wetland sites.

all	Minimum	Mean	Median	Maximum
landscape development index	1.018	2.313	2.192	4.125
nitrogen loss rate	0.180	0.500	0.500	0.781
phosphorus loss rate	0.004	0.014	0.014	0.025
natural	Minimum	Mean	Median	Maximum
landscape development index	1.018	1.375	1.367	1.609
nitrogen loss rate	0.180	0.338	0.338	0.445
phosphorus loss rate	0.004	0.007	0.007	0.010
hand-picked	Minimum	Mean	Median	Maximum
landscape development index	2.002	2.437	2.289	3.173
nitrogen loss rate	0.391	0.513	0.526	0.609
phosphorus loss rate	0.010	0.014	0.014	0.017
agricultural	Minimum	Mean	Median	Maximum
landscape development index	2.168	3.260	3.373	4.125
nitrogen loss rate	0.497	0.672	0.691	0.781
phosphorus loss rate	0.014	0.020	0.022	0.025

Water chemistry

All data were imported or entered into an MSAccess relational database. Laboratory water chemistry values labeled as non-detectable (all lead and cadmium) were divided by two (Appendix B.). *In situ* and laboratory chemistry values were averaged for each wetland (Appendix C.). The 26 sites were coded as natural (1), hand-picked (2), or agricultural (3). NCSS (Hintze 2013) was used to run summary and statistical analyses to determine if the three groups differ from each other. Table 4 and Table 5 provides summary statistics. Wetland 1309 was part of a large complex that had been drained and reduced to one pool from which we collected one water sample for laboratory chemistry. At this site hardness (392 mg/l) and its constituents (Ca = 106 mg/l, Cl = 455 mg/l, Cu = 3.96 ug/l, Mg = 30.9 mg/l, TDS = 1070 mg/l), as well as the *in situ* parameters conductivity (1.59 mS/cm), TDS (1060 mg/l), and salinity (0.08%) were extremely high. The site in Bee Hollow Conservation Area (bhca) also had high values of hardness 620 mg/l, Mg 67.5 mg/l, Ca 137 mg/l, lab TDS 890 mg/ml, conductivity 1.08 mS/cm, *in situ* TDS 691 mg/l, and salinity = 0.05%. Thus, these 8 values from 1309 and these 7 values from bhca were excluded from all statistical analyses except for descriptive statistics tables.

Table 4. Descriptive statistics for in situ water chemistry measurements by land use codes: all (n =26), natural (low human land use, n = 10), hand-picked (n = 7), and agricultural (n = 9). Cond. = conductivity, min = minimum, max = maximum. Includes outlier values from sites 1309 and bhca.

all	water temp C	pH	ORP mV	cond. mS/cm	turbidity NTU	DO mg/L	TDS g/L	salinity %
Min	21.48	6.23	-90.33	0.07	4.47	0.00	0.05	0.00
Mean	26.80	7.70	102.57	0.31	50.49	5.06	0.21	0.01
Median	26.57	7.61	111.50	0.19	32.45	4.97	0.13	0.01
Max	31.27	9.07	294.00	1.59	174.00	16.23	1.06	0.08
natural								
Min	23.39	7.19	-56.67	0.13	12.55	0.00	0.08	0.01
Mean	25.92	7.68	78.57	0.28	69.81	4.20	0.18	0.01
Median	25.04	7.53	78.17	0.24	61.35	3.47	0.16	0.01
Max	30.67	9.07	218.33	0.48	174.00	15.13	0.31	0.02
hand								
Min	21.48	6.91	-90.33	0.07	4.47	0.39	0.05	0.00
Mean	26.07	7.84	126.90	0.34	9.51	3.89	0.21	0.01
Median	26.44	7.68	170.00	0.18	7.70	3.47	0.12	0.01
Max	29.58	8.64	294.00	1.08	20.47	8.16	0.69	0.05
ag								
Min	24.86	6.23	57.00	0.07	17.64	1.44	0.06	0.00
Mean	28.36	7.61	110.31	0.33	60.89	6.91	0.24	0.02
Median	28.90	7.63	106.67	0.18	52.70	5.96	0.10	0.01
Max	31.27	8.52	172.67	1.59	164.67	16.23	1.06	0.08

Table 5. Descriptive statistics for laboratory water chemistry measurements by land use codes: all (n =26), natural (low human land use, n = 10), hand-picked (n = 7), and agricultural (n = 9) wetlands. Hard. = hardness, min = minimum, max = maximum. Includes outlier values from sites 1309 and bhca.

	Cd	Ca	Cl	Cu	Hard.	Pb	Mg	TDS	TN	TOC	TP	TSS	Zn
all	ug/L	mg/L	mg/L	ug/L	mg/L	ug/l	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	ug/L
Min	0.05	9.35	1.68	0.25	31.80	0.25	2.06	70.00	0.72	5.22	0.10	6.50	1.07
Mean	0.05	35.48	22.11	0.91	131.92	0.25	10.53	217.60	1.62	11.62	0.44	42.65	5.69
Median	0.05	27.48	3.71	0.69	92.45	0.25	5.61	143.50	1.50	10.75	0.40	40.50	1.97
Max	0.05	137.00	455.00	3.96	620.00	0.25	67.50	1070.00	3.24	21.40	0.97	115.50	79.37
natural													
Min	0.05	14.75	3.34	0.25	50.80	0.25	3.39	101.00	0.74	5.22	0.14	20.50	1.13
Mean	0.05	33.15	6.31	1.01	120.61	0.25	9.19	183.45	1.97	11.49	0.47	51.75	3.22
Median	0.05	30.23	5.00	0.92	104.28	0.25	7.04	166.75	2.00	12.03	0.47	43.00	2.45
Max	0.05	56.10	12.05	2.68	218.00	0.25	18.95	302.00	3.23	17.95	0.80	112.50	6.84
hand													
Min	0.05	9.35	1.93	0.25	31.80	0.25	2.06	70.00	0.78	8.18	0.13	6.50	1.60
Mean	0.05	43.36	3.79	0.53	173.26	0.25	15.79	248.36	1.13	11.36	0.34	13.14	14.00
Median	0.05	21.85	2.74	0.25	72.80	0.25	4.43	117.50	0.86	9.62	0.37	10.00	2.75
Max	0.05	137.00	9.51	1.25	620.00	0.25	67.50	890.00	1.71	19.30	0.51	25.50	79.37
ag													
Min	0.05	11.95	1.68	0.25	39.05	0.25	2.25	74.50	0.72	7.43	0.10	21.00	1.07
Mean	0.05	31.93	53.90	1.09	112.34	0.25	7.93	231.61	1.61	11.95	0.49	55.50	1.98
Median	0.05	27.20	3.38	0.82	89.80	0.25	4.95	139.00	1.36	10.05	0.42	49.50	1.73
Max	0.05	106.00	455.00	3.96	392.00	0.25	30.90	1070.00	3.24	21.40	0.97	115.50	4.87

Correlations with indices

We expected water TN, TP, turbidity, and TSS to be higher in agricultural wetlands due to higher loading rates from runoff, and thus to be correlated positively with NLR and PLR. However, scatter plots and linear regressions lines do not reveal strong trends (Figure 7 and Figure 8). None of the relationships between these parameters and indices were significant (all $p > 0.31$) and all were weak explanatory variables (all $R^2 < 0.413$). The lack of strong relationships between predictive variables such as reference condition groups and nutrient concentrations is well illustrated in the above scatter plot. The overlapping cloud distribution of all three groups is evident and suggest a continuum of nutrient concentrations occurs within all groups with the hand-picked group having the most restrictive high concentration cluster. This one-time snapshot of water chemistry may not be enough to reveal long-term trends associated with land use. Of the other chemistry parameters, the only significant ($p < 0.05$) correlations, which were weak, were water temperature positive with LDI ($R^2 = 0.17$) and chloride negative with NLR and PLR ($R^2 = 0.20, 0.19$ respectively).

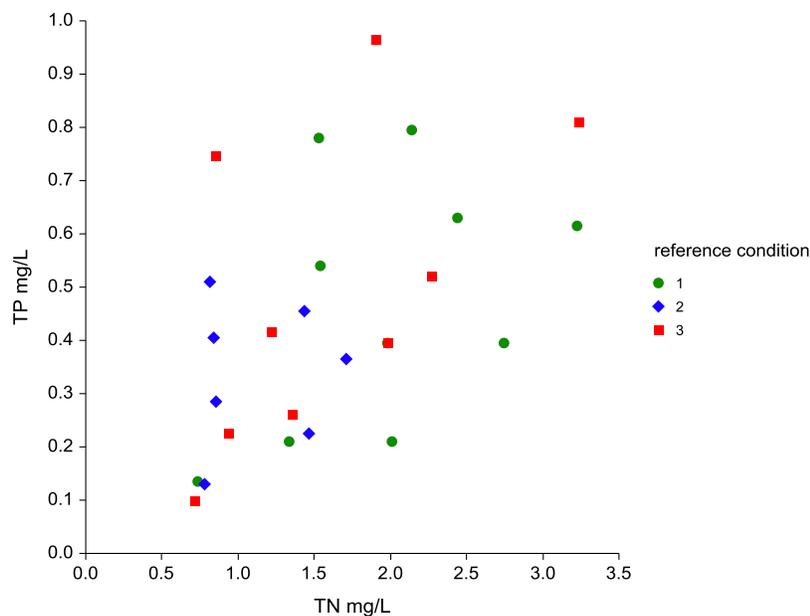


Figure 7. Scatter plot for total phosphorus (TP) versus total nitrogen (TN). Reference conditions: 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands.

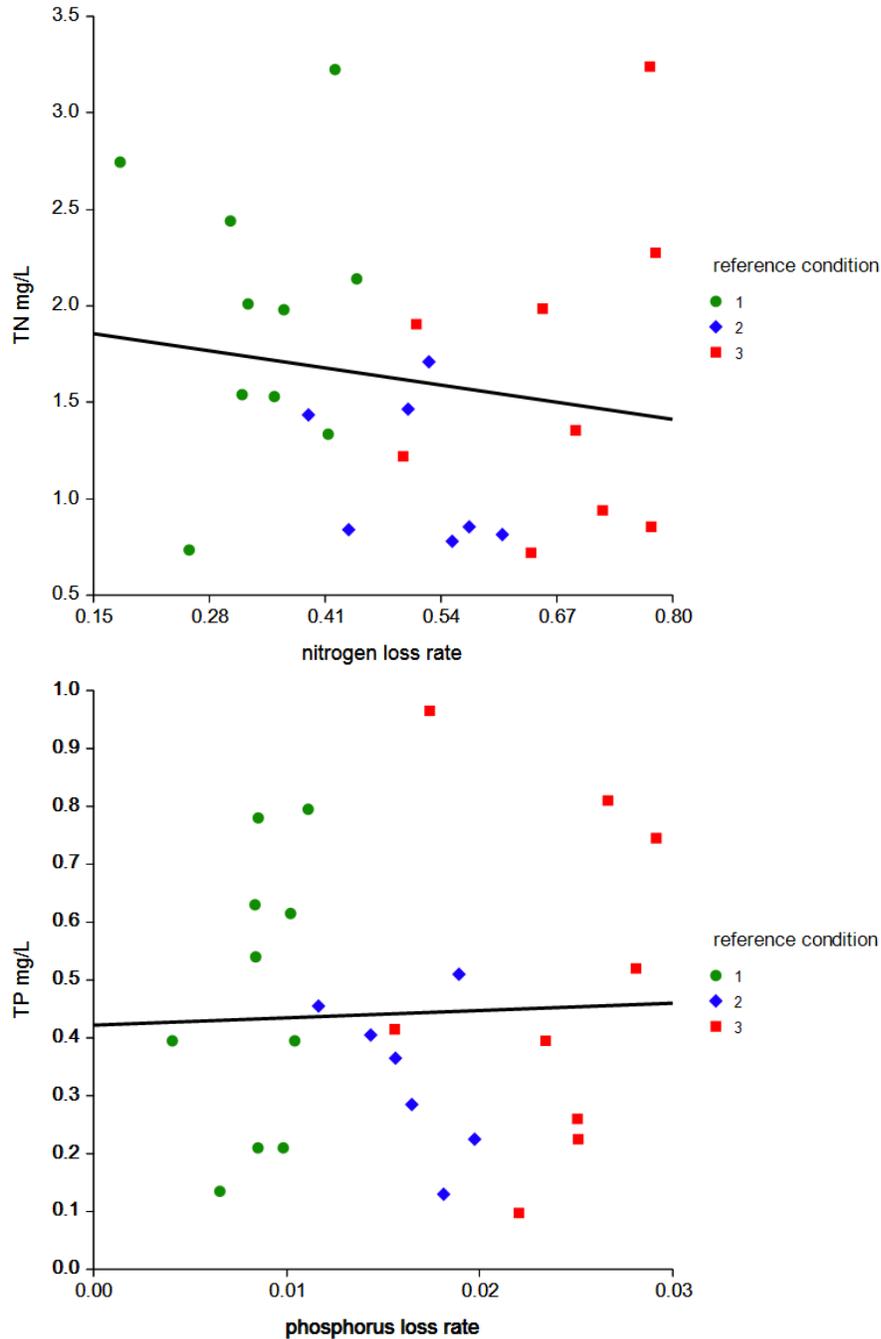


Figure 8. Scatter plots and linear regression lines for total nitrogen (TN) and total phosphorus (TP) versus nitrogen or phosphorus loss rate. TN linear regression $R^2 = 0.02$, $p = 0.45$; TP linear regression $R^2 = 0.002$, $p = 0.73$. Reference conditions: 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands.

A priori reference conditions

We examined the chemistry data to determine if the natural, agricultural, and hand-picked categories varied from one other (Figure 9 and Figure 10). One-way analysis of variance (ANOVA) shows that the means of at least two groups vary for only TSS ($p=0.01$) and turbidity

($p=0.02$), with values lower in the hand-picked sites than in sites located in either the natural or agricultural landscapes.

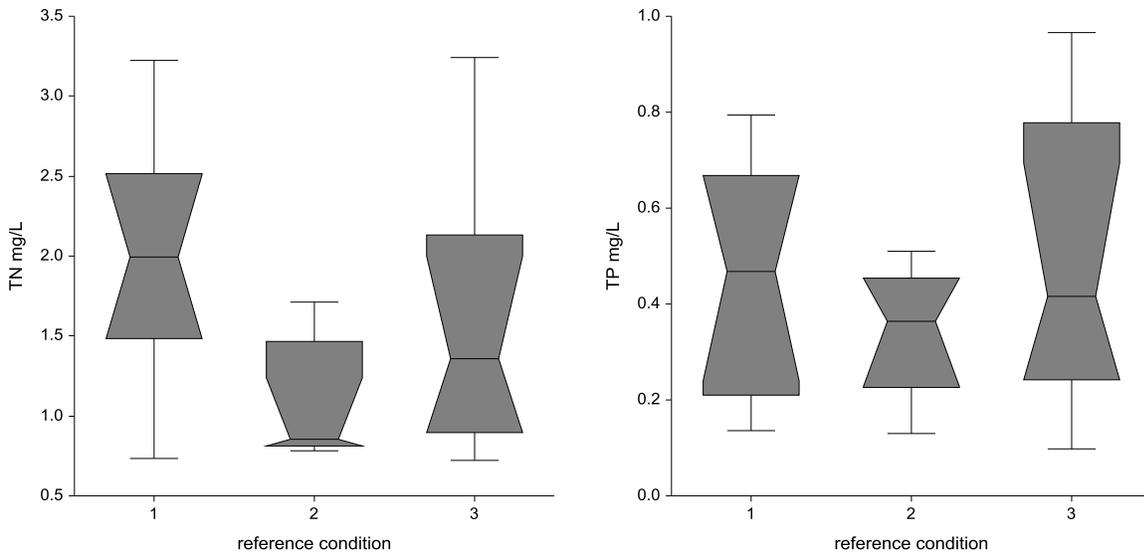


Figure 9. Notched box plots for total nitrogen and total phosphorus, by reference condition of 1 natural land use, 2 hand-picked, or 3 agricultural land use. The means do not significantly vary by category (i.e. groupings).

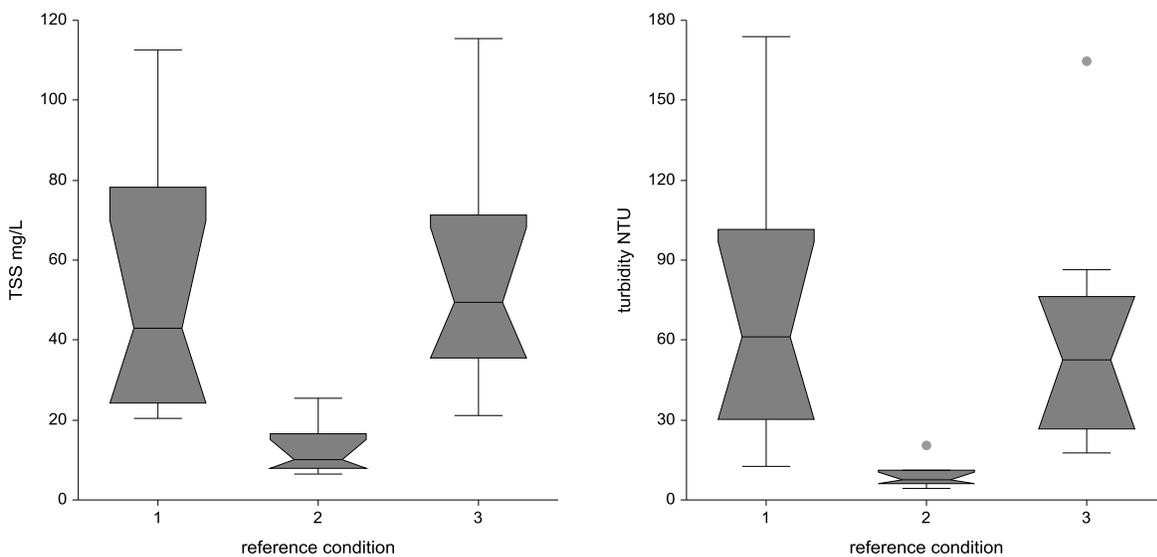


Figure 10. Notched box plots for total suspended solids (TSS) and turbidity by reference condition of 1 natural land use, 2 hand-picked, or 3 agricultural land use. The means significantly vary ($p<0.02$). Outliers are grey circles.

The notched box plots for nutrients did show that the natural site category had the highest median TN value when compared to both hand-picked and agricultural category medians, while TP medians for all categories were very similar. Why TN in the water is generally higher in natural wetlands than the other categories is not understood at this time especially in light of the predicted NLR values for this group of wetlands. The notched box plots and supporting one-way ANOVA results for group differences for TSS and turbidity, of which TSS is a part, show a that the hand-picked group had significantly lower values for these two variables. Overall the hand-picked wetland group has lower TN and TP median values and clearer water than both the natural and agricultural groups. These results run somewhat contrary to the predictive nature of the NLR and PLR.

Additional one-way ANOVAs on other measured water quality variables from just natural versus agricultural sites found few significant differences between these groupings. Natural sites were higher in Cl ($p=0.05$), TDS ($p=0.04$), Mg ($p=0.06$), total hardness ($p=0.06$), and salinity ($p=0.01$) when compared to only the agricultural group. Natural sites also had lower water temperatures ($p=0.02$), but because sampling took place throughout the summer and at different times of the day little value can be placed on this temperature difference. These same concerns apply to dissolved oxygen and pH values that also can change dramatically within a diurnal cycles. The temporal variability of dissolved oxygen in wetland waters and its rapid response to biological processes means that snapshot measurements are not particularly useful, and regular monitoring is required to understand and characterize the dissolved oxygen of any particular wetland water body.

Ecoregions

Data were examined for variations between the two ecoregions in which the sties were located, Western Corn Belt Plains (WCB) and Central Irregular Plains (CIP). One-way ANOVAs showed that the following parameters varied by ecoregion: WCB was higher in Ca ($p = 0.00$), total hardness ($p = 0.00$), Mg (0.00), TDS ($p = 0.01$), conductivity ($p= 0.00$), TDS ($p = 0.01$), and salinity ($p = 0.01$). One would expect the agricultural influence in the WCB to be higher and more prevalent, but in the selection process we were able to nearly balance the number of sites in the agricultural and natural category groupings (

Table 6).

Table 6. Number of sites in each ecoregion by site type. Western Corn Belt Plains (WCB) and Central Irregular Plains (CIP).

Ecoregion	Natural	Hand-picked	Agricultural
CIP	4	4	3
WCB	6	3	6

Macroinvertebrates

Across the study, 48 unique families of macroinvertebrates were collected, consisting of 5830 individual organisms. Chironomidae were found at all 26 sites, followed by Coenagrionidae at 24, and Baetidae at 22. Seven families were found at only one site each (Planariidae, Viviparidae (snails), Crangonyctidae (amphipods), and the insects Corduliidae, Hydrometridae, Corydalidae, and Ephydriidae). Larvae within the midge family Chironomidae were the most numerous organisms collected (1544 organisms), followed by nymphs of Baetidae (501), and Caenidae (428). Nymphs of these two mayfly families are common members of both lake, pond and stream communities being less sensitive to extreme environmental conditions than most other mayflies. Chironomidae is composed of a very large number of genera and species that range from sensitive to very tolerant of environmental impacts.

Across all the study sites 118 taxa were collected. Taxa found at the most number of sites were the midge *Polypedilum illinoense* group (22 sites), Callibaetis (21 sites), Tubificidae (20 sites) and *Caenis* (19 sites). The most numerous taxa were the mayflies *Callibaetis* (501 organisms) and *Caenis* (428), followed by the isopod Caecidotea (349) and oligochaete Tubificidae (332).

Fourteen indices were calculated in EcoMeas 1.6 (2005, Appendix D.). Only those that differed statistically between site categories are discussed within this report. None of the community indices were found to vary between the two ecoregions. Unexpectedly macroinvertebrate abundance (number of organisms) was found to increase with land use indices indicative of increased agriculture suggesting that perhaps increased nutrient levels among other agricultural-related changes increase the standing crop (i.e. abundance/sample) of invertebrates. Linear regression showed that abundance increases with all three land use indices: LDI ($R^2 = 0.28$, $p = 0.01$), NLR ($R^2 = 0.27$, $p = 0.01$) and PLR ($R^2 = 0.29$, $p = 0.00$). Also one-way ANOVAs on reference condition type (natural, hand-picked, or agricultural) showed that agricultural sites scored higher in the following macroinvertebrate community indices: Gleason diversity ($p=0.04$), Margalef diversity ($p=0.04$), taxa richness ($p=0.03$), and taxa abundance ($p=0.00$) (Figure 11). The Tukey-Kramer post-hoc test was used to see if there were differences between means of two groups. Taxa richness differed only between hand-picked and agricultural groups ($p = 0.04$). Taxa abundance differed between the natural and agricultural groups ($p = 0.00$).

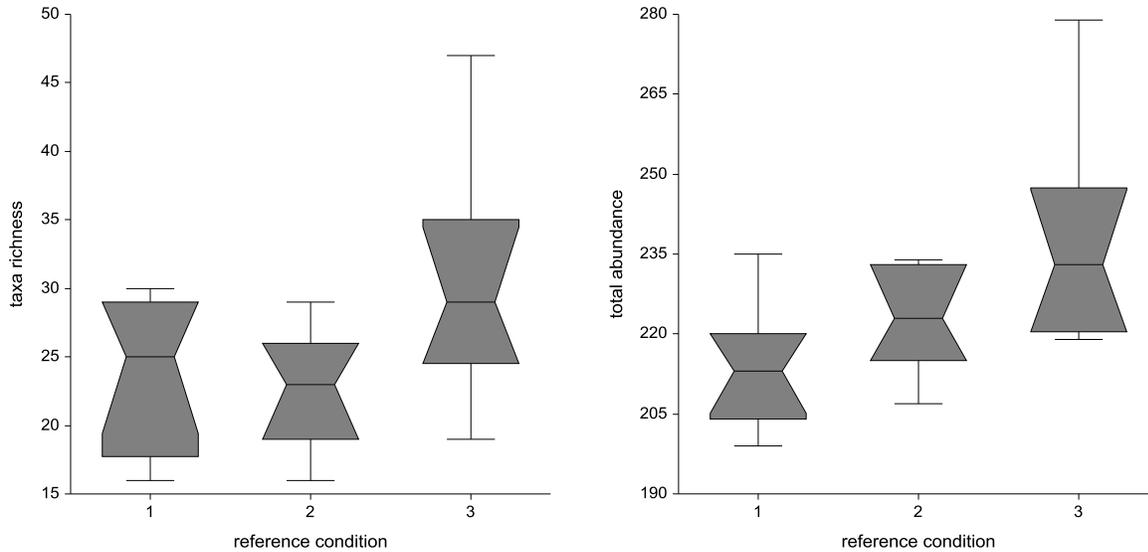


Figure 11. Notched box plots for taxa richness and abundance in wetlands coded by reference condition of 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands. Agricultural sites had a significantly higher mean taxa richness than the hand-picked sites ($p = 0.04$). Agricultural sites had a significantly higher mean total abundance than the natural sites ($p = 0.00$).

Further examination by scatter plot shows site 1309, where there was just one small pool of water, had the highest total abundance, 279 organisms in the sample (Figure 12). However, taxa richness (24) at this site was approximately half that of the richest site (Figure 13). The highest taxa richness (47) was also at an agricultural site, 2810, which had the highest Gleason and Margalef diversity index values. As Gleason and Margalef are calculated similarly and correlate with taxa richness ($R^2=1.0$), we focused our continuing analytical assessments on taxa richness.

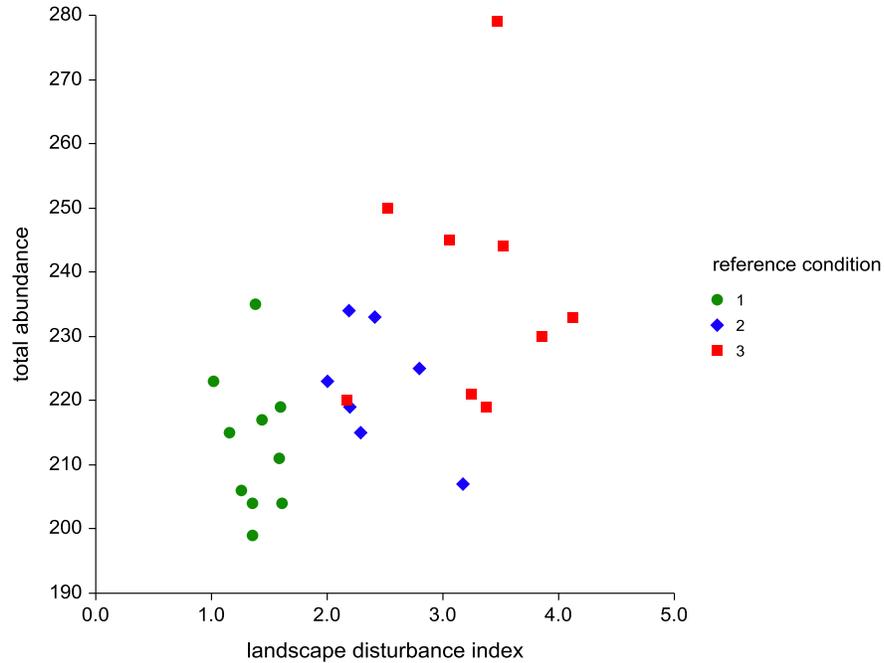


Figure 12. Scatter plot for total abundance versus landscape disturbance index. Reference conditions: 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands. Highest taxa abundance (279) was found at site 1309 which is classified as an agricultural site.

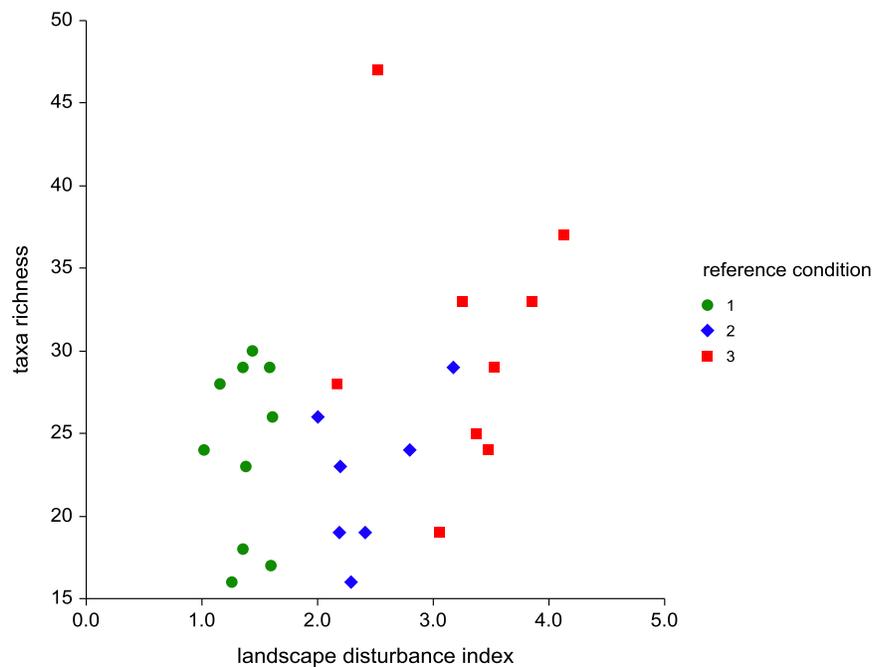


Figure 13. Scatter plot for taxa richness versus landscape disturbance index. Reference conditions: 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands. Highest taxa richness (47) was found at site 2810 which is classified as an agricultural site.

The high abundance and taxa richness in the agricultural sites was somewhat unexpected at least in regards to overall richness. Examination of taxa distribution reveals that high numbers of certain taxa at a few agricultural sites contributed to the agricultural sites having higher abundance (Table 7). The most numerous organism collected at agricultural sites was the mayfly genus *Caenis*, comprising 10.2% of the organisms, and found at 7 of the 9 sites. It was found at the same number of natural sites, yet in fewer numbers (6%). *Hyaella azteca*, an amphipod that is sensitive to aquatic contaminants yet tolerant to wide ranges of DO, alkalinity, sediment grain size, and organic matter content (Wang et al. 2004), was the second most numerous organism at agricultural sites, comprising 8.8 % of the organisms. It was found at almost twice as many natural sites (7) though much fewer in numbers, comprising 2% of the organisms found there. Of the 4 agricultural sites that had *H. azteca*, one had 158 organisms (site 1628) thus skewing results. *Tanypus*, a non-biting midge found at equal numbers of agricultural sites and natural sites (5 each), comprised 8% of the organisms at agricultural sites, and 3% at natural sites. At one agricultural site (1309) 106 organisms were found, skewing results.

Table 7. Number and percent of organisms comprising the most numerous taxa collected in the study wetlands, by land use classification of natural, hand-picked, or agricultural. For comparison are shown are the numbers of those taxa in other land use categories

natural			hand picked			agricultural		
taxon	#	%	taxon	#	%	taxon	#	%
<i>Trichocorixa</i>	203	9.5	Caecidotea	336	21.6	<i>Caenis</i>	218	10.2
Tubificidae	195	9.1	<i>Callibaetis</i>	171	11	<i>Hyaella azteca</i>	188	8.8
<i>Palaemonetes kadiakensis</i>	193	9.0	<i>Polypedilum illinoense</i> gr.	136	6.35	<i>Tanypus</i>	171	8.0
<i>Caenis</i>	124	6	<i>Caenis</i>	86	5.53	Trichocorixa	97	4.53
<i>Hyaella azteca</i>	36	2	<i>Hyaella azteca</i>	48	3.08	Tubificidae	108	5.04
<i>Tanypus</i>	62	3	<i>Tanypus</i>	1	0.06	<i>Palaemonetes kadiakensis</i>	5	0.23

Trichocorixa was the most numerous organism in natural sites, consisting of 9.5% of the organisms and found at 4 sites. One of these sites (1647) had 138 individuals. It was also found at 4 agricultural sites (and 2 hand-picked). Tubificidae, comprising 9.1% of the organisms at the natural sites, was found at 9 natural and 6 agricultural sites (and 5 hand-picked). The numerous natural sites explains its high numbers. *Palaemonetes kadiakensis*, Mississippi grass shrimp, was found outside its International Union for Conservation of Nature (IUCN) reported range (Degraeve and Rogers 2013) at 5 natural sites which had 20 – 74 organisms, while only a total of 5 individuals were found at agricultural sites (i.e. 2 out of 9 sites).

Ephemeroptera, Plecoptera, and Trichoptera richness have been used as indicators of disturbance in lotic water, with various metrics of these three orders summarized in Table 7-1 of Barbour et al. (1999). In our study EPT richness or abundance did not clearly separate the natural from the agricultural sites. As expected for wetlands, there were no Plecoptera at any site as these organisms require high dissolved oxygen levels to complete their immature life cycles. Wetlands typically have relatively low dissolved oxygen (DO) concentrations and high DO fluxes due to naturally high biological oxygen demand (BOD and abundance of macrophytes (see Rose and Crumpton 1996). Trichoptera were found at 3 natural (10 organisms, genera *Oecetis* and *Ochrotrichia*) and 2 agricultural sites (2 specimens, one *Oecetis* and one *Oxyethira* nymph). Collectively Ephemeroptera immatures were found at all natural sites (310 organisms or 14%), all agricultural sites (362 organisms or 17%), and 6 of 7 hand-picked sites (257 organisms or 17%). All mayfly nymphs were either species with the genera *Caenis* or *Callibaetis* and co-occurred within all three study categories.

Vegetation

Plants identified at each site were categorized by wetland indicator status (obligate, facultative, etc.), and provenance (native or non-native). The online Universal Floristic Quality Assessment (FQA) Calculator (<http://universalfqa.org>, Freyman et al. 2016) was used to assign Missouri coefficients of conservatism to species and calculate the floristic quality index of each site (Appendix E.). The Floristic Quality Index, also called the Floristic Quality Assessment Index (FQAI), has been shown to have value in identifying wetland condition (Herman 2001, Lopez and Fennessy 2002, Miller and Wardrop 2006). Lopez and Fennessy (2002) found that wetlands and the immediate zone around them tended to have lower FQAI scores and were dominated by plants associated with heavy agricultural and urban watersheds. For example, these authors found that for their study wetlands (n=20) in Ohio, FQAI (=FQI) scores for fairly high quality wetlands were typically 25 or higher. The FQI also performed well in discriminating wetland condition in the Great Lakes coastal wetlands and consistently outperformed coefficient of conservatism indices in this capacity (Bourdaghs et al. 2006). These authors found that the performance results of indices that included or excluded introduced species (our all and native only FQIs) were nearly the same but they thought conceptually that introduced species should be included in the FQI, arguing that “introduced species are simultaneously a source of and a response to anthropogenic stress.”

We hypothesized that:

1. Natural sites would have more native and obligate wetland species than agricultural sites.
2. Agricultural sites would have more introduced species.
3. Floristic quality index would be higher in natural sites.

The following vegetation indices were calculated (based on all species, natives only, or non-natives only, Table 8):

- Species richness – Total number of species.
- Mean conservatism coefficient (C) – Larger numbers represent more species restricted to higher quality areas.
- Wetness coefficient – The USFWS wetland indicator values (e.g. UPL, FACW, OBL, etc.) converted to numbers for use in the FQI calculator. Smaller numbers represent more obligate wetland species.
- Floristic quality index (FQI) – Mean coefficient of conservatism \times square root of number of species.
- Adjusted FQI – Native mean C divided by 10 and multiplied by the square root of the native species richness divided by total species richness and multiplied by 100. To reduce sensitivity to species richness and include the contribution of non-native species when assessing sites with high levels of human disturbance, the Adjusted FQI was developed (Miller and Wardrop 2006).

Table 8. Descriptive statistics of the vegetation indices calculated for the wetlands, by land use codes: all (n =26), natural (n = 10), hand-picked (n = 7), and agricultural (n = 9) wetland sites.

Rich = richness, conserve = conservatism coefficient, FQI = floristic quality index, adj. = adjusted.

all	total rich	native rich	non-native rich	% native	% non-native	mean conserve.	native mean conserve.	total FQI	native FQI	adj. FQI	mean wetness	native mean wetness
Min	20	19	0	0	79	2.5	2.8	14.5	15.3	27.2	-4.6	-4.7
Mean	38	35	3	8	92	3.1	3.4	18.6	19.5	32.4	-2.9	-3.2
Median	35	33	3	8	92	3.0	3.2	18.4	19.1	31.0	-2.8	-3.2
Max	63	55	10	21	100	4.0	4.3	26.2	27.2	41.3	-1.7	-2.1
natural												
Min	20	19	0	83	0	2.6	2.8	15.2	15.7	27.2	-4.6	-4.7
Mean	31	29	2	94	6	3.1	3.3	17.0	17.4	32.0	-3.3	-3.4
Median	32	30	2	95	5	3.0	3.2	16.5	17.3	30.4	-3.3	-3.5
Max	48	44	4	100	17	3.9	4.1	18.7	19.2	40.0	-2.4	-2.7
hand												
Min	24	23	1	79	2	2.8	3.2	14.5	15.3	30.2	-4.3	-4.3
Mean	33	30	3	91	9	3.4	3.8	19.4	20.4	35.9	-3.1	-3.4
Median	27	24	2	92	8	3.4	3.6	18.3	20.6	35.7	-3.2	-3.5
Max	53	52	6	98	21	4.0	4.3	26.2	27.2	41.3	-2.0	-2.1
ag												
Min	25	23	2	82	4	2.5	2.9	16.2	17.3	27.4	-2.8	-3.2
Mean	50	44	6	90	10	2.8	3.2	19.7	21.1	30.1	-2.3	-2.7
Median	50	47	7	89	11	2.7	3.1	19.1	20.8	28.3	-2.5	-2.6
Max	63	55	10	96	18	3.3	3.6	22.6	25.0	34.5	-1.7	-2.4

We examined relationships and patterns among the vegetation indices, reference condition, and other data. ANOVAs revealed that the means of all plant indices except percent natives and total FQI statistically differ by reference condition ($p < 0.05$, Table 9). The post-hoc Tukey-Kramer test verified which groups differ. Natural and hand-picked sites did not differ from each other, but means of agricultural sites differed from natural and hand-picked sites for most indices, with the trend of being higher than the other groups (Figure 14). The exception was that for the adjusted FQI score, the hand-picked group was significantly higher than the agricultural group ($p = 0.017$) but not higher than the natural category ($p = 0.534$) (Figure 15). Mean wetness condition was higher in agricultural sites than either other category, but for this index the higher values suggest that less of the wetland plant communities of agricultural wetlands were comprised of obligate wetland taxa as opposed to those of hand-picked and natural wetlands (Figure 16). Linear regression of mean wetness with LDI ($R^2 = 0.27$ $p = 0.006$) further supports that the number of obligate species decreased as agriculture increased (Figure 16). The only other significant strong regression with LDI was an increase in nonnative richness ($R^2 = 0.33$, $p = 0.002$) as agriculture increased (Figure 17).

Table 9. Summary of one-way ANOVA p values and Tukey-Kramer post hoc tests that show from which reference condition group agricultural wetlands differed for various vegetation indices. * p value < 0.05 , ** p value < 0.01 . Solid gray cells indicate no differences.

Vegetation index	ANOVA p value	natural	hand-picked
total richness	**	**	**
native richness	**	**	*
nonnative richness	**	**	*
% native species			
mean conservatism	*		**
native mean conservatism	*		*
total FQI			
native FQI	*	*	
adjusted FQI	*		*
mean wetness	**	**	*
native mean wetness	*	**	

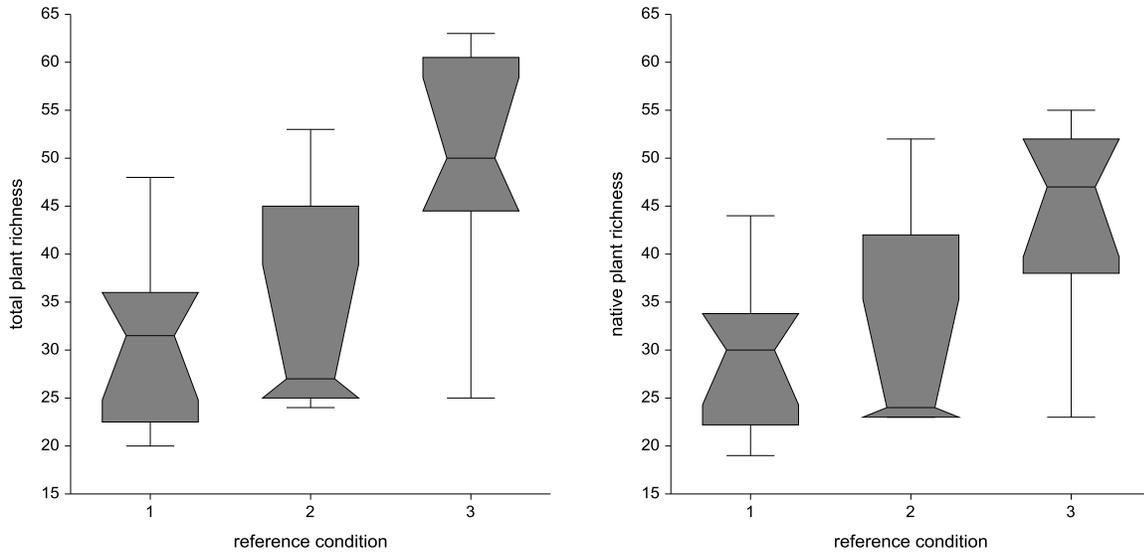


Figure 14. Notched box plots for total plant richness ($p = 0.001$) and native plant richness ($p = 0.006$) in wetlands coded by reference condition of 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands. The agricultural mean for both indices is higher than both the natural ($p \leq 0.009$) and hand-picked ($p \leq 0.02$) means of the respective index.

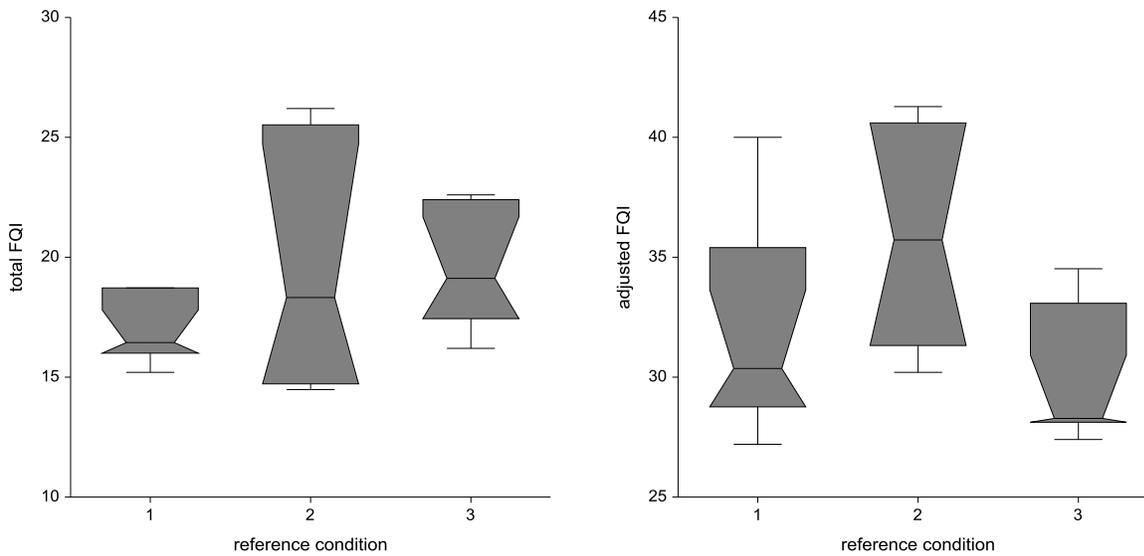


Figure 15. Percent total FQI ($p=0.126$, left) and adjusted FQI ($p=0.021$, right) by reference condition: 1 = natural, 2 = hand-picked, 3 = agricultural wetlands. Adjusted FQI scores are significantly higher in the hand-picked group than the agricultural group ($p = 0.017$).

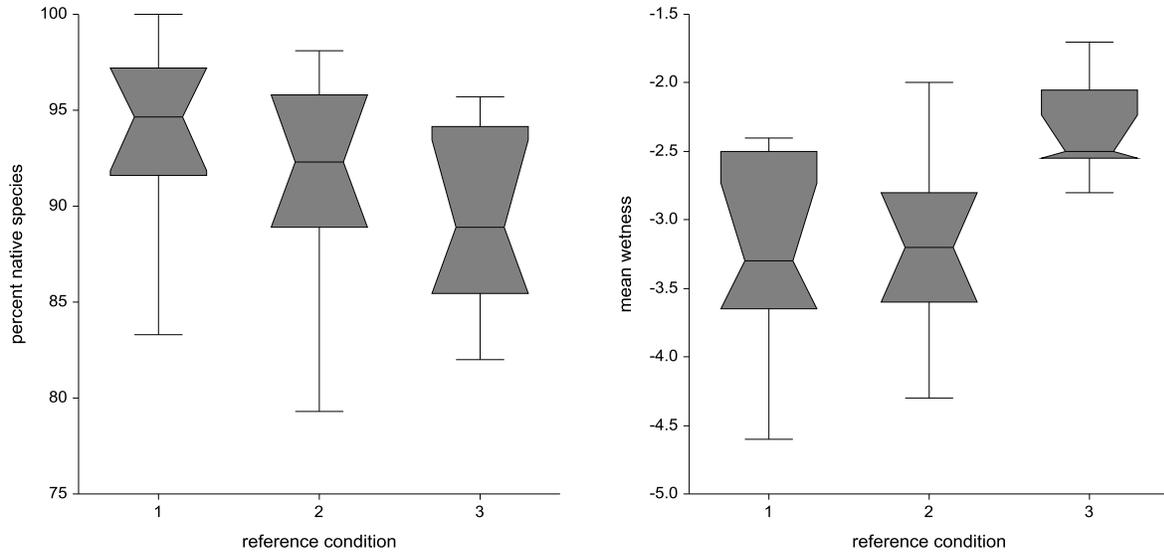
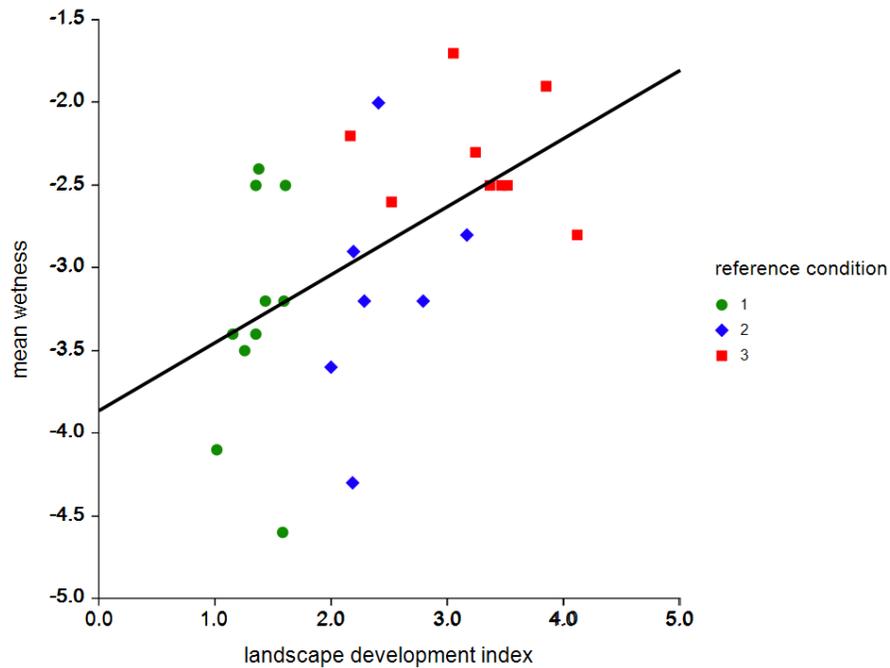


Figure 16. Notched box plots for percent native plant richness ($p = 0.205$) and mean wetness ($p = 0.006$) in wetlands coded by reference condition of 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands. The agricultural mean for mean wetness is higher than both the natural ($p = 0.007$) and hand-picked ($p = 0.038$) means, indicating fewer obligate taxa.



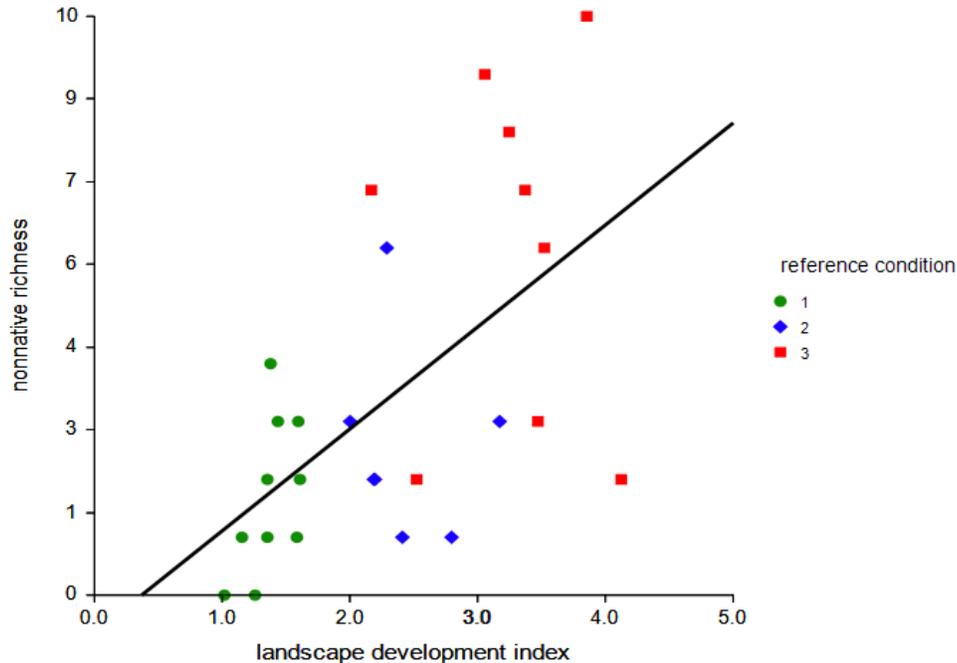


Figure 17. Scatter plots and linear regression lines for mean wetness ($R^2 = 0.27$, $p = 0.006$) and nonnative richness ($R^2 = 0.33$, $p = 0.002$) versus landscape development index. Reference conditions: 1 = natural, 2 = hand-picked, and 3 = agricultural wetlands.

A potential source of sample bias in the FQA was that each site was sampled only once. The strength of FQA is a function of having a thorough inventory of all the species at a site. A one-time visit provides just a snapshot of the vegetation. If a guild of species is largely unrepresented in a sample, such as early-flowering/early-fruiting species or late-flowering/late-fruiting species, it could affect the index. Disturbed sites typically have more non-native species, and the native species that do occur in them have lower coefficients of conservatism. Rare species, and species with higher coefficients of conservatism, if part of the under sampled guilds, could affect the indices. A potential example is the genus *Carex* (sedge). The more conservative, early-flowering species were not found at any of the sites. The species found usually were later-blooming generalists. And, unlike many forbs in vegetative conditions, it is difficult if not impossible to identify many of the species of *Carex* in vegetative condition, which means they were excluded from surveys and consequently, could have resulted in lower indices than expected.

Discussion

We expected agricultural and natural sites, as defined by land development index (LDI), to differ in water chemistry, macroinvertebrates, and plants. However, the only meaningful relationship we found was that agricultural sites had fewer obligate wetland plant species than either natural or hand-picked wetlands, which could be related to the isolation of the

agricultural wetlands. Also, the only variables that showed statistically significant (but weak) linear regressions with LDI, NLR, or PLR were water temperature, chlorine, and macroinvertebrate abundance. These regressions do not have biological significance, especially considering that the measurements were taken only once. Examining whether the study values fell into the range of values found in other studies will tell us if there are anomalies within the study group.

Previously for the USEPA Region 7 Regional Technical Assistance Group (R7 RTAG) we compiled total nitrogen, total phosphorus, and turbidity data from wetlands sampled in Kansas, Missouri, Oklahoma, and Nebraska. Five sources of data (KBS, Iowa DNR, Kansas DHE, Nebraska DEQ, USGS) contributed data collected from 1994-2008 from over 700 sampling events at 265 sites located in nine Level 3 ecoregions (see summary document https://biosurvey.ku.edu/sites/biosurvey.ku.edu/files/docs/cpcb/workgroups/nutrient/2008Nov_wetlandRTAG.pdf). Six sites in this Missouri study were in the same wetland complexes as nine sites in the R7 RTAG database (Table 10). The small sample sizes makes it difficult to evaluate variations between datasets, thus we averaged the data across ecoregions (only for sites located in Missouri), which results in more similarity between datasets (Table 11).

Table 10. Comparison of water quality values from wetland sites sampled in this study (MDNR code) and sites in the same wetland complex sampled in previous studies (R7 code). TN = total nitrogen, TP = total phosphorus.

Waterbody	Approximate distance (m) between sites	MDNR code	Average of 2 values			R7 code	Averages or single values			# records
			TN mg/l	TP mg/l	turbidity NTU		TN mg/l	TP mg/l	turbidity NTU	
Squaw Cr. NWR Pelican Pool	230	scnw	1.44	0.455	20.47	7102	1.61	0.271	145.30	1
Squaw Cr. NWR Mallard Marsh	160	2207	2.01	0.210	31.77	7462	1.45	0.594	21.00	2
Squaw Cr. Eagle Pool	1030	2092	1.53	0.780	12.55	7460	1.78	3.710	18.00	1
Squaw Cr. Eagle Pool	1340	2092	1.53	0.780	12.55	7152	2.76	1.645	277.00	1
Little Bean CA - Bean Lake	170	lbca	1.71	0.365	6.27	7128	1.03	0.150	15.86	47
Cooley Lake	250	123	0.94	0.225	26.77	7440	0.89	0.336	10.00	2
Swan Lake	420	slnw	0.78	0.130	10.33	7105	1.67	0.496	241.00	1
Swan Lake	420	slnw	0.78	0.130	10.33	7160	4.42	0.995	80.30	1
Swan Lake	1090	slnw	0.78	0.130	10.33	7464	0.74	0.149	54.00	1

Table 10 illustrates both the limited number of values available for study and the broad variance that can occur in nutrient and turbidity data. When we examined the overall mean percent differences between TN, TP and turbidity for values listed for the nine wetland waterbodies in these two database we see that mean database values can be highly different. The overall mean percent differences by variable between R7 and MDNR code database means were: 36% for TN, 68% for TP, and 84% for turbidity. These differences are most like due to temporal variations of real values and not sampling errors. Even within larger but more general wetland datasets we sometimes observed large differences in nutrient and turbidity values.

Table 11. Comparison of water quality values from wetland sites sampled in this study (MDNR) to wetlands in Missouri sampled in previous studies (R7), by ecoregions Central Irregular Plains (CIP) and Western Corn Belt Plains (WCB). TN = total nitrogen, TP = tot

MDNR	Averages			# records	R7	Averages			# records
	TN mg/l	TP mg/l	turbidity NTU			TN mg/l	TP mg/l	turbidity NTU	
CIP	1.47	0.405	57.00	11	CIP	1.44	0.364	220.64	45
WCB	1.73	0.471	45.71	15	WCB	1.16	0.315	44.75	65

In addition to the lack of meaningful water quality relationships found, no strong relationships were observed in either the macroinvertebrates or plants. While the agricultural group had a higher mean wetness index than the other two groups, indicating that it had fewer obligate wetland plant species, this could be due to the isolated nature of the agricultural sites which tended to be small and surround by crop fields. One is more likely to find obligate wetland species at sites that are large, connected to other wetlands, and less impacted by humans than at small, isolated, human-impacted sites. Many of the sites that we perceived to be of higher quality fell into the former category, while lower quality sites tended to conform to the latter (Figure 18).



Figure 18. Natural site 2518 located in a wetland complex in Fountain Grove Conservation Area (left). Contrast with Agricultural site 2382 surrounded by crop fields (right).

Only adjusted FQI scores seemed to discern some group differences where the hand-picked group had higher scores (Figure 15). It is unclear why hand-picked wetlands scored higher in this since these sites were selected based on mostly qualitative factors and BPJ (best professional judgment) which are hard to quantify and are not very repeatable unless done by the same group of experts. It may be that the Native FQI is a better indirect measure of plant species traits and overall richness that are known factors related to wetland resilience to disturbance (Engelhardt and Kadlec 2001). The fact that species richness alone was not discriminatory may be related to the lack of trait information including disturbance tolerance values and that this is just a single descriptor of the plant community (Pausas and Austin 2001). Species richness and composition (e.g. diversity) may be more dependent of hydrological regimes than water quality as shown by Pollock and co-workers (Pollock et al. 1998).

We ran hierarchical cluster analyses (Group Average, Unweighted Pair-Group, Euclidian Distance) to see which variables influenced wetland sites to group by reference condition. See Figure 19 through Figure 23 for results and interpretations.

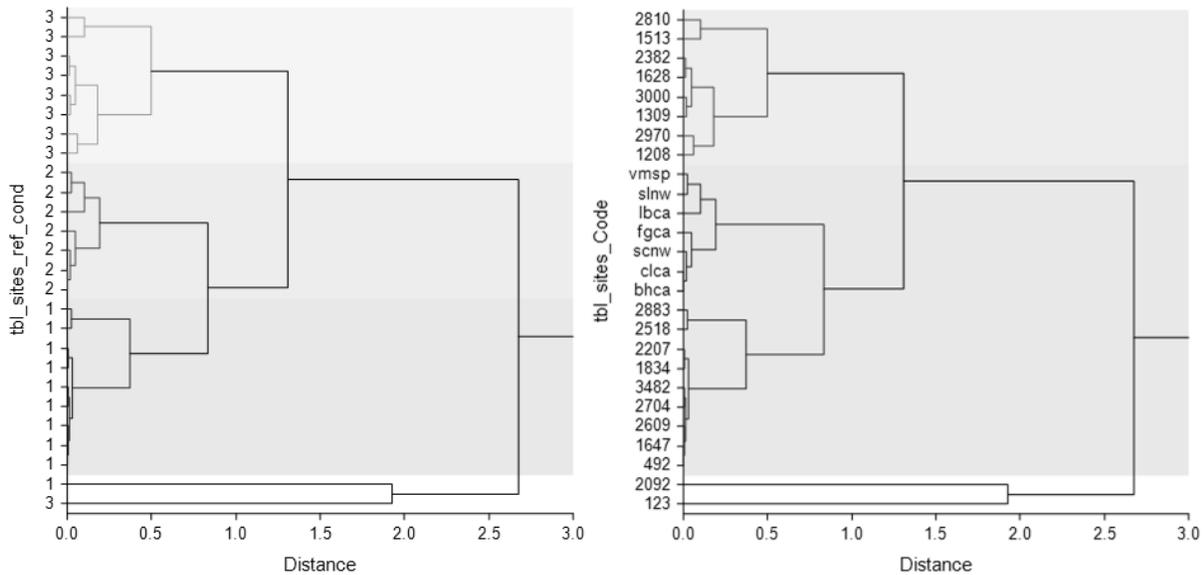


Figure 19. Dendrogram of wetland size (acres) shows clustering by the three reference condition groups: 1 = natural, 2 = hand-picked, and 3 = agricultural. Site codes are provided in the dendrogram on the right. Only two wetlands did not cluster (one natural and one agricultural). The separation of the three study groups by size may be impacting the plant indices.

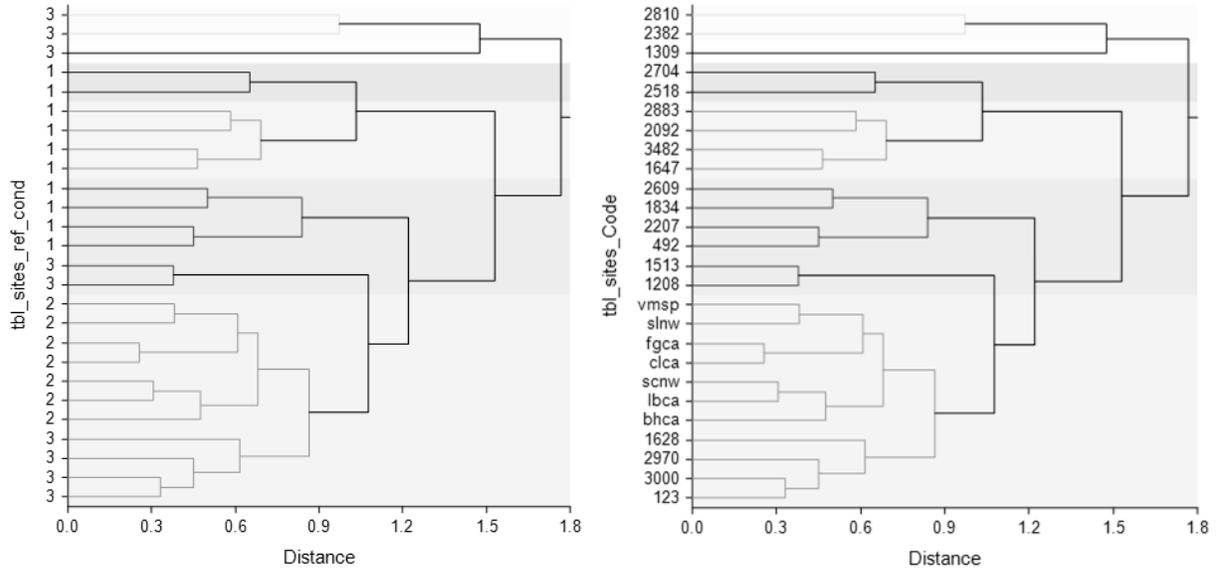


Figure 20. Dendrogram of nutrients (TN, TP) shows six clusters with mixed membership of the three reference condition groups: 1 = natural, 2 = hand-picked, and 3 = agricultural (on left, by site code on right). The largest cluster was composed of hand-picked and agricultural wetlands while the natural wetlands formed three separate clusters. Two small clusters were made up of only agricultural wetlands and one agricultural wetland failed to cluster with any other wetlands. It appears that nutrient levels in and of themselves only loosely follow our study groupings except at a larger scale.

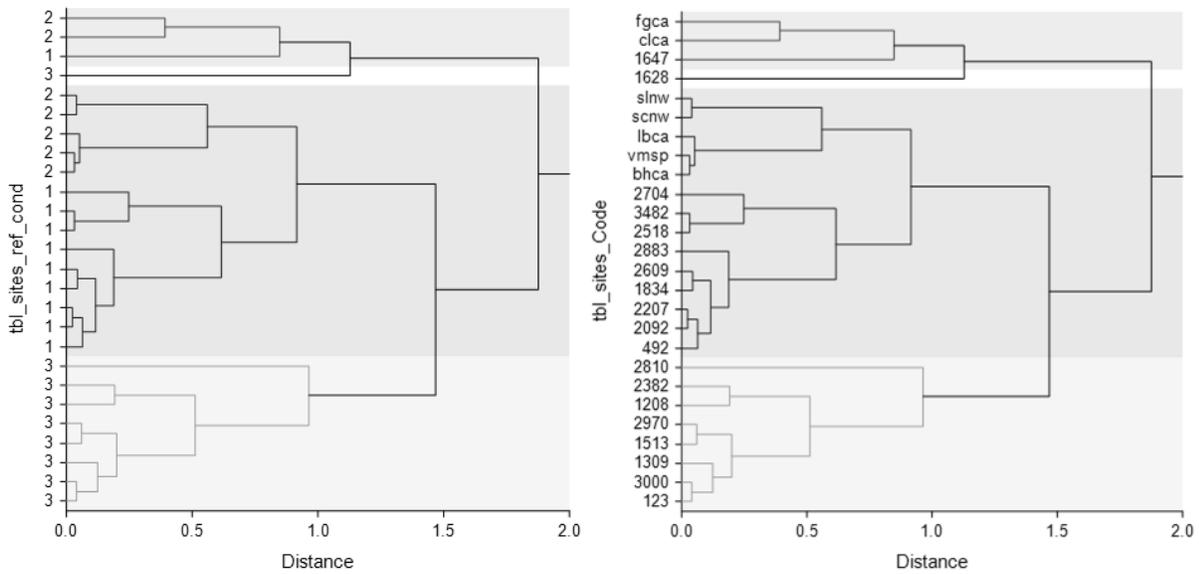


Figure 21. Dendrogram of Shannon's diversity index values for macroinvertebrates shows three clusters, on the right by reference condition (1 = natural, 2 = hand-picked, and 3 = agricultural), on the left by site code. The largest cluster had mixed membership – all natural wetlands and five of the seven hand-picked wetlands. The smallest cluster also was of mixed membership (natural and hand-picked). The last cluster was composed of all agricultural wetlands suggesting that macroinvertebrate diversity could be a useful indicator in discriminating impaired wetlands.

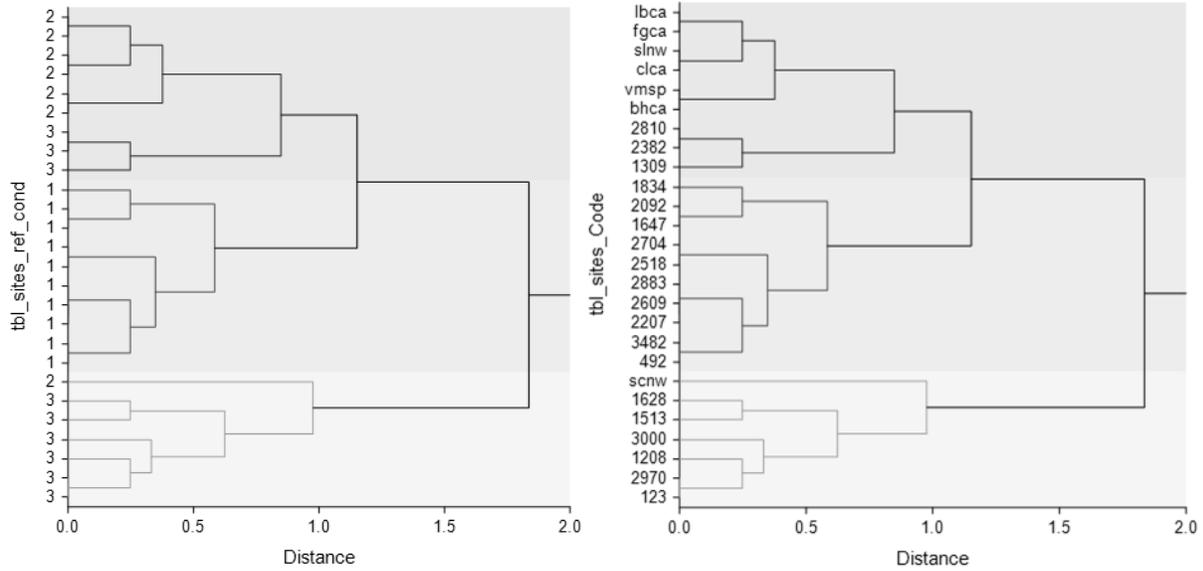


Figure 22. Dendrogram of non-native plants shows three distinct clusters on the right by reference condition (1 = natural, 2 = hand-picked, and 3 = agricultural), on the left by site code. Two clusters have only agricultural and hand-picked wetland memberships. All natural wetlands linked tightly into a single cluster suggesting that the natural wetland had very different values for non-native plant composition at least when compared the mixed ag/hand-picked clusters.

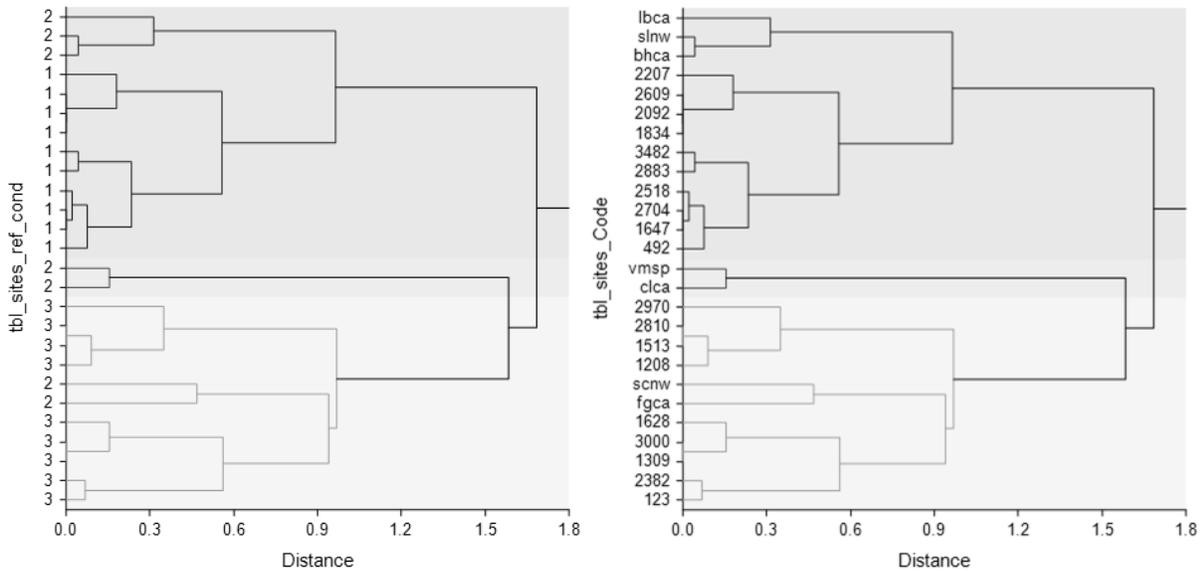


Figure 23. Dendrogram of total FQI values shows three clusters, on the right by reference condition (1 = natural, 2 = hand-picked, and 3 = agricultural), on the left by site code. One cluster is very small, with two hand-picked wetlands. The other two clusters sharply separated the natural from the agricultural wetlands with three or less hand-picked wetlands within each. The FQI scores for the measured plant community separated the natural wetlands from those located in agricultural watersheds.

A factor that might have been complicating the identification of land use variables and wetland health (water quality, plant and macroinvertebrate metrics) relationships could be related to how we describe and measure land use variables of interest. Perhaps nutrient loading alone or as measured as a watershed-wide variable is not appropriate. Houlahan and co-investigators (Houlahan et al. 2006) noted that adjunct land use (250 -300 m buffer) and wetland size were very important in predicting land use effects on wetlands. Lopez and Fennessy (2002) did find the FQI (=FQAI) valuable in identifying a disturbance gradient in Ohio depressional wetlands, but was not correlated to either wetland size or water quality. Lastly, it has been more recently shown that single metrics such as richness or even the multifaceted FQI may not be able to discern wetland impacts without further adaptations to their structure as predictive indicators to wetland disturbance (see Matthews et al. 2005, Miller and Wardrop 2006). More recently investigators have developed multi-metric indices that have value in assessing wetland conditions but most are regional in nature (Miller et al. 2016).

In conclusion we found a few, but weak, meaningful relationships between our wetland indicators and the land use metrics that were developed as potential indicators of landscape disturbance and nutrient enrichment. Further work is required to understand what hand-picked wetlands mean and what existing or new water quality or biological metrics may be of value in determining water quality influences on wetland condition in the presence of other co-occurring disturbances (e.g. altered hydrological conditions, fragmentation). Pausas and Austin (2001) point out those disturbances may express themselves in multiple ways depending upon the nature of the disturbances (e.g. frequency, intensity, season and extent) and thus generalizations about impacts and their indicators are difficult. Different disturbance regimes or different moments after disturbance may express themselves differently making the identification of universal (in time and space) difficult. Because of the natural dynamics of both wetlands and potential disturbances to them we suggest that long-term studies are needed to identify changes in water quality with changes in wetland bio-integrity within either natural or disturbed landscapes. Changes in hydrological regimes may, in fact, mask changes in wetland biota due to just changes in water quality.

References

- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.
- Blackwood, M.A. 2007. Standard Operating Procedure for the Benthic Macroinvertebrate Laboratory, Kansas Biological Survey, Central Plains Center for BioAssessment. 89pp.

Brown, M.T., and M.B. Vivas. 2005. Landscape Development Intensity Index. *Environmental Monitoring and Assessment*. 101:289-309.

De Grave, S. and C. Rogers. 2013. *Palaemonetes kadiakensis*. The IUCN Red List of Threatened Species 2013: e.T197722A2497462. Retrieved 31 July 2017 from <http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T197722A2497462.en>.

EcoMeas 1.6. 2005. Kansas Biological Survey. Retrieved 1 Aug. 2017 from http://biosurvey.ku.edu/sites/biosurvey.ku.edu/files/docs/cpcb/data_resources/databases/ecomeas01_6.mdb.

Engelhardt, K.A. and J.A. Kadlec. 2001. Species traits, species richness and the resilience of wetlands after disturbance. *J. Aquatic Plant Manage.* 39:36-39.

EPA (2002). *Methods for Evaluating Wetland Condition: Land-Use Characterization for Nutrient and Sediment Risk Assessment*. Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-R-02-025.

Freyman, W.A., L.A. Masters, and S. Packard. 2016. The Universal Floristic Quality Assessment (FQA) Calculator: an online tool for ecological assessment and monitoring. *Methods in Ecology and Evolution*. 7(3):380–383.

Hintze, J.L. 2013. Number Cruncher Statistical System: Dr. Jerry L. Hintze, Kaysville, Utah.

Huggins, D., M. Moffet. 1988. Proposed biotic and habitat indices for use in Kansas streams. Kansas Biological Survey, Lawrence, KS Report No. 35. 128 pp.

Homer, C.G., Dewitz, J.A., Yang, L., Jin, S., Danielson, P., Xian, G., Coulston, J., Herold, N.D., Wickham, J.D., and Megown, K. 2015. Completion of the 2011 National Land Cover Database for the conterminous United States-Representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing*. 81(5):345-354.

Houlahan, J.E., P.A. Keddy, K. Makkay, and C.S. Findlay. 2006. The effects of adjacent land use on wetland species richness and community composition. *Wetlands* 26:79–96.

Jenson, S.K., and J.O. Domingue. 1988. Extracting Topographic Structure from Digital Elevation Data for Geographic Information System Analysis. *Photogrammetric Engineering & Remote Sensing*. 54(11):1593-1600.

Kastens, J.H. 2008. Some New Developments on Two Separate Topics: Statistical Cross Validation and Floodplain Mapping. Doctoral Dissertation, 191 pp. Dept. of Mathematics, University of Kansas. Ann Arbor: ProQuest/UMI, 2008. (Publication No. 3316028.) Retrieved from <http://hdl.handle.net/1808/5354>.

Kriz, J., D. Huggins, C. C. Freeman, J. Kastens. 2007. Assessment of floodplain wetlands of the Lower Missouri River using a reference-based study approach. Kansas Biological Survey, Lawrence, KS Report No. 142. 63 pp.

Lopez, R.D. and M.S. Fennessy. 2002. Testing the floristic quality assessment index as an indicator of wetland condition. *Ecological Applications* 12:487-497.

Matthews J.W., P.A. Tessene, S.M. Wiesbrook, and B.W. Zercher. 2005. Effect of area and isolation on species richness and indices of floristic quality in Illinois, USA wetlands. *Wetlands* 25(3):607-615.

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.

Miller K.W., B.R. Mitchell, and B.J. McGill. 2016. Constructing multimetric indices and testing ability of landscape metrics to assess condition of freshwater wetlands in the Northeastern US. *Ecological Indicators* 66:143–152.

Pausas, J.G. and M.P. Austin. 2001. Patterns of plant species richness in relation to different environments: An appraisal. *J. Vegetation Sci.* 12:153-166.

Pollock, M.M., R.J. Naiman, and T.A. Hanley. 1998. Plant species richness in riparian wetlands—a test of biodiversity theory. *Ecology* 79:94-105.

Rose, C. and W.G. Crumpton. 1996. Effects of emergent macrophytes on dissolved oxygen dynamics in a prairie pothole wetland. *Wetlands* 16(4):495-502.

USEPA. 1995. Environmental Monitoring and Assessment Program (EMAP): Laboratory Methods Manual – Estuaries, Volume 1: Biological and Physical Analyses. EPA/620/R-95/008. U.S. Environmental Protection Agency, Narragansett, RI.

USEPA. 2004. Wadeable Stream Assessment: Benthic Laboratory Methods. EPA841- B-04-007. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, D.C.

USEPA. 2009. Core elements of an effective state and tribal wetlands program. 42pp. Retrieved 2 Oct. 2017 from https://www.epa.gov/sites/production/files/2015-10/documents/2009_03_10_wetlands_initiative_cef_full.pdf.

Wang, F, R.R. Goulet, and P.M. Chapman. 2005. Testing sediment biological effects with the freshwater amphipod *Hyalella azteca*: The gap between laboratory and nature. *Chemosphere* 57(11):1713-24.

Williams, B., E. D'Amico, J.H. Kastens, J. Thorp, J. Flotemersch, and M. Thoms. 2013. Automated riverine landscape characterization: GIS-based tools for watershed-scale research, assessment, and management. *Environmental Monitoring and Assessment*. 185:7485-7499. DOI: 10.1007/s10661-013-3114-6.

Appendix A. Study sites, with reference condition (ref cond) of 1 natural land use, 2 hand-picked, or 3 agricultural land use. Polygon refers to the polygon code in associated shapefiles. National wetland inventory classification (NWI class), ecoregion (ecoreg) Central Irregular Plains (CIP) or Western Cornbelt Plains (WCB), landscape disturbance index (LDI250m), nitrogen loss rate (nlr250m), phosphorus loss rate (plr250m), and area in acres.

site	poly-gon	name	ref cond	NWI class	ecoreg	county	latitude	longitude	date	ldi250m	nlr250m	plr250m	acres
0123	123	Cooley Lake	3	PEMKh	WCB	Clay	39.24990	-94.23314	18-Aug-2016	3.37	0.72	0.02	357.85
0492	492	Hicklin Lake	1	PUBG	WCB	Lafayette	39.19039	-93.78904	18-Aug-2016	1.61	0.36	0.01	12.10
1208	1208	Cut-Off Lake	3	PEMCd	WCB	Chariton	39.36318	-93.04205	15-Aug-2016	3.25	0.65	0.02	41.21
1309	1309	private	3	PUBFx	WCB	Chariton	39.49990	-93.17615	16-Aug-2016	3.47	0.77	0.02	21.89
1513	1513	private	3	PEMAd	WCB	Chariton	39.39528	-93.09393	15-Aug-2016	3.86	0.78	0.02	101.27
1628	1628	private	3	PUBG	WCB	Andrew	39.88357	-94.78004	18-Aug-2016	3.06	0.64	0.02	10.93
1647	1647	Thurnau CA	1	PEMC	WCB	Holt	40.17673	-95.44465	3-Sep-2015	1.60	0.30	0.01	12.05
1834	1834	Ideker Farm levee	1	PEM/SS1C	WCB	Holt	40.20248	-95.47661	14-Sep-2015	1.38	0.26	0.01	18.37
2092	2024	Squaw Creek NWR - Eagle Pool	1	PEMKFh	WCB	Holt	40.07253	-95.23412	3-Sep-2015	1.44	0.35	0.01	533.28
2207	2207	Squaw Creek NWR - Mallard Marsh	1	PEMKCh	WCB	Holt	40.09373	-95.27313	3-Sep-2015	1.59	0.32	0.01	17.69
2382	2382	private	3	PUBGh	CIP	Livingston	39.74588	-93.37128	16-Aug-2016	4.12	0.78	0.03	13.11
2518	2627	Fountain Grove CA	1	PUBKh	CIP	Linn	39.72218	-93.34246	2-Sep-2015	1.26	0.42	0.01	78.72
2609	2609	Fountain Grove CA	1	PEMA	CIP	Chariton	39.69974	-93.26910	2-Sep-2015	1.16	0.41	0.01	11.26
2704	2704	Fountain Grove CA	1	PEMA	CIP	Chariton	39.69822	-93.28107	2-Sep-2015	1.02	0.18	0.00	13.20
2810	2729	Swan Lake NWR - Silver Lake	3	PEMKAh	WCB	Chariton	39.61612	-93.14806	16-Aug-2016	2.52	0.51	0.02	118.55
2883	5002	Swan Lake NWR - Silver Lake	1	PFO1KCh	CIP	Chariton	39.63106	-93.15868	1-Sep-2015	1.35	0.32	0.01	75.16
2970	2989	Scobee Lake	3	PUBF	CIP	Adair	40.30518	-92.70725	17-Aug-2016	2.17	0.50	0.01	51.47
3000	3000	private	3	PEMCd	CIP	Putnam	40.58849	-92.72389	17-Aug-2016	3.52	0.69	0.02	19.30
3482	3482	private	1	PUBKx	WCB	Chariton	39.58075	-93.24192	16-Aug-2016	1.35	0.45	0.01	14.29
bhca	5003	Bee Hollow CA	2	PEMFd	CIP	Macon	39.61607	-92.51314	12-Aug-2015	3.17	0.50	0.02	1.15
clca	5004	Chloe Lowry Marsh CA	2	PUBF	CIP	Mercer	40.43419	-93.61208	11-Aug-2015	2.41	0.61	0.02	1.23
fgca	2313	Fountain Grove CA	2	PUBKh	CIP	Linn	39.73476	-93.34686	11-Aug-2015	2.19	0.44	0.01	10.06
lbca	15	Little Bean Marsh CA	2	PEMF	WCB	Platte	39.49974	-95.02689	10-Aug-2015	2.20	0.53	0.01	48.34
scnw	5001	Squaw Creek NWR- Pelican Pool	2	PEMKCh	WCB	Holt	40.07172	-95.26514	10-Aug-2015	2.29	0.39	0.01	4.13
slnw	5101	Swan Lake NWR - Swan Lake	2	PEM	CIP	Chariton	39.61818	-93.23405	11-Aug-2015	2.80	0.55	0.02	28.82
vmsp	5102	Van Meter State Park	2	PEM	WCB	Saline	39.26830	-93.27234	12-Aug-2015	2.00	0.57	0.01	32.81

Appendix. B. Descriptive statistics for laboratory water chemistry measurements, averaged from two samples at each site except for site 1309 which had one sample. Hard. = hardness, min = minimum, max = maximum.

site	Cd ug/L	Ca mg/L	Cl mg/L	Cu ug/L	Hardness mg/L	Pb ug/L	Mg mg/L	TDS mg/L	TN mg/L	TOC mg/L	TP mg/L	TSS mg/L	Zn ug/L
0123	0.05	31.70	3.69	0.25	97.70	0.25	4.50	139.00	0.94	7.43	0.23	30.00	1.07
0492	0.05	44.70	10.14	0.53	169.50	0.25	14.00	240.50	1.98	11.45	0.39	40.50	1.13
1208	0.05	31.25	2.39	0.87	115.90	0.25	9.23	154.50	1.98	11.65	0.39	41.00	1.96
1309	0.05	106.00	455.00	3.96	392.00	0.25	30.90	1070.00	3.24	21.40	0.81	79.00	1.57
1513	0.05	27.20	3.29	1.74	89.80	0.25	5.32	138.00	2.27	10.05	0.52	115.50	4.87
1628	0.05	21.95	5.75	0.25	75.20	0.25	4.95	100.50	0.72	7.89	0.10	21.00	1.12
1647	0.05	47.30	5.13	2.67	179.00	0.25	14.70	250.50	2.44	12.60	0.63	82.00	1.60
1834	0.05	56.10	12.05	1.12	218.00	0.25	18.95	302.00	0.73	5.22	0.14	24.50	1.20
2092	0.05	38.40	8.77	0.25	144.50	0.25	11.90	224.00	1.53	14.25	0.78	20.50	3.21
2207	0.05	32.25	6.97	0.38	114.05	0.25	8.12	169.00	2.01	7.89	0.21	23.00	5.62
2382	0.05	11.95	3.17	1.06	39.05	0.25	2.25	74.50	0.86	9.78	0.75	63.50	2.07
2518	0.05	28.20	4.26	0.91	94.50	0.25	5.90	164.50	3.23	17.95	0.62	77.00	1.75
2609	0.05	22.35	3.34	1.52	73.70	0.25	4.34	113.50	1.33	7.34	0.21	51.50	3.14
2704	0.05	14.75	3.73	1.20	50.80	0.25	3.38	101.00	2.75	9.76	0.39	112.50	1.42
2810	0.05	13.90	6.73	0.39	49.90	0.25	3.68	105.50	1.90	14.30	0.97	46.00	1.95
2883	0.05	21.05	4.87	0.92	71.60	0.25	4.63	127.00	1.54	13.50	0.54	45.50	6.26
2970	0.05	15.70	3.38	0.44	55.65	0.25	4.00	144.50	1.22	15.65	0.42	49.50	1.73
3000	0.05	27.75	1.68	0.82	95.85	0.25	6.50	158.00	1.36	9.39	0.26	54.00	1.44
3482	0.05	26.35	3.89	0.55	90.40	0.25	5.96	142.50	2.14	15.00	0.79	40.50	6.83
bhca	0.05	137.00	2.74	0.25	620.00	0.25	67.50	890.00	1.47	19.30	0.23	9.00	5.02
clca	0.05	14.90	1.93	0.97	48.75	0.25	2.81	86.50	0.81	8.78	0.51	10.00	5.15
fgca	0.05	9.35	2.67	1.25	31.80	0.25	2.06	70.00	0.84	9.62	0.40	8.00	2.13
lbca	0.05	61.95	3.69	0.25	214.50	0.25	14.50	277.50	1.71	12.65	0.36	16.50	2.75
scnw	0.05	10.80	3.41	0.25	40.00	0.25	3.17	84.00	1.44	11.95	0.45	16.50	79.37
slnw	0.05	21.85	9.51	0.47	72.80	0.25	4.43	117.50	0.78	8.18	0.13	6.50	1.59
vmisp	0.05	47.65	2.61	0.25	185.00	0.25	16.05	213.00	0.86	9.04	0.28	25.50	1.97

Appendix C. Descriptive statistics for in situ water chemistry measurements, averaged from three measurements at each site, except for site 1309 which had one measurement. ORP = oxygen reduction potential, tds = total dissolved solids.

site	water temp C	pH	ORP mV	conductivity mS/cm	turbidity NTU	dissolved oxygen mg/L	TDS g/L	salinity %
0123	29.00	7.63	120.00	0.19	26.77	3.52	0.13	0.01
0492	24.95	7.54	-0.33	0.35	33.13	1.80	0.24	0.02
1208	27.13	8.35	106.67	0.25	17.64	7.51	0.40	0.01
1309	29.45	8.52	170.00	1.59	86.50	16.23	1.06	0.08
1513	28.71	8.43	172.67	0.15	164.67	10.68	0.10	0.01
1628	28.90	7.63	86.67	0.16	52.63	5.82	0.10	0.01
1647	30.67	9.07	124.67	0.38	127.33	15.13	0.24	0.02
1834	23.39	7.93	218.33	0.48	26.10	6.04	0.31	0.02
2092	24.95	7.49	-14.33	0.41	12.55	1.13	0.27	0.02
2207	24.88	7.52	148.67	0.27	31.77	0.36	0.17	0.01
2382	31.27	6.23	130.50	0.07	52.70	5.04	0.06	0.00
2518	24.60	7.19	25.00	0.22	93.03	0.00	0.14	0.01
2609	26.06	7.46	184.00	0.16	77.27	5.09	0.11	0.01
2704	26.70	7.59	116.33	0.13	174.00	3.76	0.08	0.01
2810	25.91	6.79	57.00	0.31	26.73	1.44	0.07	0.01
2883	27.83	7.46	40.00	0.18	77.43	5.52	0.11	0.01
2970	29.98	7.20	82.00	0.10	54.17	5.96	0.07	0.00
3000	24.86	7.74	67.33	0.18	66.20	6.01	0.13	0.01
3482	25.13	7.54	-56.67	0.18	45.43	3.19	0.12	0.01
bhca	24.70	8.64	-15.67	1.08	11.07	1.19	0.69	0.05
clca	21.48	7.64	83.00	0.18	6.30	0.39	0.06	0.00
fgca	26.44	7.68	185.67	0.07	4.47	6.89	0.05	0.00
lbca	27.97	7.10	294.00	0.43	6.27	4.90	0.28	0.02
scnw	28.51	6.91	261.67	0.10	20.47	3.47	0.06	0.00
slnw	29.58	8.30	170.00	0.18	10.33	8.16	0.12	0.01
vmisp	23.81	8.61	-90.33	0.34	7.70	2.26	0.23	0.02

Appendix D. Macroinvertebrate indices calculated in EcoMeas.

Site	Brillouin's Index	Fager's Number of Moves	Gleason's Index	Margalef's Index	McIntosh's Index	Menhinick's Index	Richness: Abundance	Shannon's Index (H')	Simpson's Compliment	Simpson's Index	Simpson's Reciprocal	Standard Deviation	Taxa Richness	Total Abundance
0123	1.01	890	10.68	4.45	0.69	1.69	0.11	1.08	0.88	0.12	8.24	13.06	25	219
0492	1.09	231	11.26	4.70	0.76	1.82	0.13	1.18	0.92	0.08	11.91	9.12	26	204
1208	1.15	746	14.08	5.93	0.76	2.22	0.15	1.24	0.92	0.08	12.07	9.30	33	221
1309	0.99	-442.5	9.81	4.08	0.62	1.44	0.09	1.05	0.83	0.17	5.93	20.96	24	279
1513	1.06	504	13.97	5.88	0.70	2.18	0.14	1.15	0.88	0.12	8.52	12.26	33	230
1628	0.58	521	7.95	3.27	0.36	1.21	0.08	0.63	0.56	0.44	2.29	35.86	19	245
1647	0.54	603	7.26	2.97	0.36	1.15	0.08	0.58	0.56	0.44	2.29	33.74	17	219
1834	1.05	82	9.70	4.03	0.72	1.50	0.10	1.12	0.90	0.10	9.84	12.47	23	235
2092	1.08	371.5	12.84	5.39	0.74	2.04	0.14	1.16	0.91	0.09	10.68	10.23	30	217
2207	1.07	-37	12.48	5.23	0.72	2.00	0.14	1.15	0.90	0.10	9.74	10.73	29	211
2382	1.20	-561	15.63	6.60	0.78	2.42	0.16	1.30	0.93	0.07	13.94	8.57	37	233
2518	0.92	4	6.91	2.82	0.69	1.11	0.08	0.98	0.87	0.13	7.91	13.88	16	206
2609	1.05	29.5	12.00	5.03	0.72	1.91	0.13	1.13	0.90	0.10	9.54	11.20	28	215
2704	0.84	-1051.5	10.22	4.25	0.60	1.61	0.11	0.90	0.81	0.19	5.18	18.31	24	223
2810	1.35	-730	19.60	8.33	0.84	2.97	0.19	1.47	0.96	0.04	23.07	5.93	47	250
2883	1.12	-121	12.56	5.27	0.77	2.03	0.14	1.21	0.92	0.08	12.95	8.38	29	204
2970	1.05	632	11.95	5.01	0.71	1.89	0.13	1.13	0.89	0.11	8.88	12.05	28	220
3000	1.02	-501	12.15	5.09	0.68	1.86	0.12	1.10	0.87	0.13	7.74	14.45	29	244
3482	0.93	-117.5	7.83	3.21	0.69	1.28	0.09	0.99	0.87	0.13	7.94	13.20	18	199
bhca	1.04	408	12.52	5.25	0.70	2.02	0.14	1.12	0.89	0.11	8.76	11.33	29	207
clca	0.43	1063	8.03	3.30	0.26	1.24	0.08	0.47	0.44	0.56	1.77	39.36	19	233
fgca	0.55	1268	8.02	3.30	0.32	1.24	0.08	0.60	0.51	0.49	2.03	36.71	19	234
lbca	1.03	5	9.83	4.08	0.72	1.55	0.11	1.10	0.90	0.10	9.78	11.71	23	219
scnw	0.88	379.5	6.86	2.79	0.64	1.09	0.07	0.93	0.84	0.16	6.36	17.44	16	215
slnw	0.86	576.5	10.20	4.25	0.59	1.60	0.11	0.92	0.80	0.20	5.03	18.81	24	225
vmsp	1.04	-799.5	11.07	4.62	0.72	1.74	0.12	1.11	0.89	0.11	9.42	11.94	26	223

Appendix E. Vegetation indices. Rich = richness, coeff. = coefficient, FQI = floristic quality index.

site	total rich	native rich	nonnative rich	% native species	% nonnative	mean conservatism coeff.	native mean conservatism coeff.	total FQI	native FQI	adjusted FQI	mean wetness	native mean wetness
0123	42	35	7	94.3	5.7	2.5	3.0	16.2	17.7	27.4	-2.5	-3.2
0492	35	33	2	83.3	16.7	2.8	3.0	16.6	17.2	29.1	-2.5	-2.7
1208	63	55	8	87.3	12.7	2.8	3.2	22.2	23.7	29.9	-2.3	-2.7
1309	50	47	3	94.0	6.0	2.7	2.9	19.1	19.9	28.1	-2.5	-2.6
1513	61	51	10	83.6	16.4	2.9	3.5	22.6	25.0	32.0	-1.9	-2.4
1628	50	41	9	82.0	18.0	2.6	3.1	18.4	19.8	28.1	-1.7	-2.4
1647	34	31	3	91.2	8.8	2.8	3.1	16.3	17.3	29.6	-3.2	-3.6
1834	48	44	4	91.7	8.3	2.7	2.9	18.7	19.2	27.8	-2.4	-2.7
2092	39	36	3	92.3	7.7	3.0	3.2	18.7	19.2	30.7	-3.2	-3.5
2207	21	20	1	95.2	4.8	3.9	4.1	17.9	18.3	40.0	-4.6	-4.7
2382	25	23	2	92.0	8.0	3.3	3.6	16.5	17.3	34.5	-2.8	-3.0
2518	29	29	0	100.0	0.0	3.0	3.0	16.2	16.2	30.0	-3.5	-3.5
2609	27	26	1	96.3	3.7	3.6	3.7	18.7	18.9	36.3	-3.4	-3.5
2704	23	23	0	100.0	0.0	3.4	3.4	16.3	16.3	34.0	-4.1	-4.1
2810	47	45	2	95.7	4.3	3.3	3.5	22.6	23.5	34.2	-2.6	-2.6
2883	20	19	1	95.0	5.0	3.4	3.6	15.2	15.7	35.1	-3.4	-3.4
2970	60	53	7	88.3	11.7	2.7	3.0	20.9	21.8	28.2	-2.2	-2.6
3000	54	48	6	88.9	11.1	2.6	3.0	19.1	20.8	28.3	-2.5	-3.0
3482	35	33	2	94.3	5.7	2.6	2.8	15.4	16.1	27.2	-2.5	-2.7
bhca	27	24	3	88.9	11.1	2.8	3.2	14.5	15.7	30.2	-2.8	-3.3
clca	53	52	1	98.1	1.9	3.5	3.6	25.5	26.0	35.7	-2.0	-2.1
fgca	26	24	2	92.3	7.7	4.0	4.3	20.4	21.1	41.3	-4.3	-4.3
lbca	25	23	2	92.0	8.0	3.2	3.5	16.0	16.8	33.6	-2.9	-3.1
scnw	29	23	6	79.3	20.7	3.4	4.3	18.3	20.6	38.3	-3.2	-3.7
slnw	24	23	1	95.8	4.2	3.0	3.2	14.7	15.3	31.3	-3.2	-3.5
vmssp	45	42	3	93.3	6.7	3.9	4.2	26.2	27.2	40.6	-3.6	-3.7