

COMPARING CREATED AND NATURAL DEPRESSIONAL WETLANDS  
THROUGH TROPHIC ANALYSIS OF MACROINVERTEBRATES

A Thesis submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

by

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Shante N. Eisele ENTITLED Comparing Created and Natural Depressional Wetlands Through Trophic Analysis of Macroinvertebrates BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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## ABSTRACT

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Comparing Created and Natural Depressional Wetlands Through Trophic Analysis of  
Macroinvertebrates.

Macroinvertebrates are important contributors to wetland ecosystems due to their role in decomposition, nutrient cycling, and as a food resource for other organisms. Several studies have analyzed the macroinvertebrate communities in created wetlands, but few have evaluated them in the context of trophic structure in both created and natural wetlands. The objective of this study is to better understand benthic macroinvertebrate community composition and trophic structure in created and natural wetlands. My central hypotheses were that macroinvertebrate communities in created wetlands would have (1) differing composition and (2) less complex trophic structure with shorter food-chain length compared to natural wetlands. Macroinvertebrates and soil cores were collected from five created and two natural depressional marshes. I assessed macroinvertebrate community characteristics such as diversity and composition, and functional feeding group composition. I used stable isotope analysis to determine food-chain length and other trophic metrics. Soil cores were used to determine bulk density, texture, and the C:N profile of the soil in the wetlands. Through a combination of univariate (e.g. ANOVA) and multivariate analyses (e.g. NMDS, PERMANOVA) these conclusions were met: (1) Macroinvertebrate taxa composition differed statistically between wetland types ( $p= 0.05$ ); (2) FCL did not differ significantly between wetland types. In addition, functional feeding group composition was trending toward significance ( $p = 0.095$ ), and soils were found to be distinct between wetland

types ( $p= 0.043$ ), with bulk density being a strong driver of that relationship ( $p= 0.012$ ). These results show that in these wetlands, macroinvertebrate species present are different, however the overall function they provide are very similar between wetland types. The habitat characteristics in created wetlands that are known to quickly develop (e.g. plant community composition) were similar to the natural wetlands, but characteristics that take longer (e.g. soil bulk density) were still distinct between types.

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## I. INTRODUCTION

Wetlands provide important ecological services, such as flood protection, stabilization of sediments, and filtration of nutrients and pollution (Mooney et al., 2005). However, these benefits were largely unappreciated when an estimated 53% of the wetlands in the continental U.S. were drained for agricultural and urban development between 1780 and 1980 (Dahl, 1980). While this is both a national and global problem, it is an especially important issue in the Midwestern U.S. where more than 90% of wetlands have been drained in Ohio specifically (Mitsch, 1992). In response to widespread loss across the country, and in growing recognition of the importance of wetlands, the U.S. government introduced the Clean Water Act (CWA) in 1972.

The CWA specifies that restoration of degraded wetlands or construction of new wetlands may be required to mitigate wetland loss (Olson, 1992). The process of restoring, creating, enhancing or preserving aquatic resources to offset any adverse impacts that may occur during construction is referred to as compensatory mitigation (CWA Section 404). Although not stated explicitly in the CWA, the objective is not just preservation of the wetland areal base, but preservation of the important ecosystem services that wetlands provide (Uhl, 2011).

It is uncertain whether mitigation provides functional preservation when a new wetland is constructed (hereafter created wetland). Extensive effort has been made to study the differences between created and natural wetlands because of the requirement of



compensatory mitigation when planning development of a wetland (Campbell et al., 2002; Marchetti et al., 2010; Spadafora et al., 2016). While many created wetlands have the physical appearance of natural wetlands, such as standing water and some wetland vegetation, they can lack important wetland plant species and soil structure. Most importantly, created wetlands are often deficient in many essential functions (e.g. carbon storage, nutrient cycling) (Campbell et al., 2002; Hossler et al., 2011; Moreno-Mateos et al., 2012).

One important component of wetlands is the macroinvertebrate community, which contributes to many important processes such as decomposition of litter, nutrient cycling, and regulation of primary production (Balcombe et al., 2005). Macroinvertebrates also play a large role in the food web, as their abundance and diversity make them accessible to other invertebrates, as well as fish, amphibians, and waterfowl, as primary food sources (Balcombe et al., 2005). Macroinvertebrates are also one of the first groups of organisms to populate a new system, a necessary step precluding successful colonization of other species that prey on them. Thus, they are certainly an important component in conservation efforts (Stanczak, 2004).

Wetlands have the potential to support complex macroinvertebrate communities that consist of primary consumers as well as predators (Culler et al., 2013). Heterogeneous habitats—including macrophytes, riparian vegetation, soil, and detritus—encourage the development of diverse benthic macroinvertebrate communities (Sartori et al., 2015, Brraich & Kaur, 2017). There are two primary ways in which plant communities can impact macroinvertebrates, food source and habitat. Created wetlands have been shown to have lower primary production (Hossler et al., 2011) which can mean

a less abundant food source provided by the plant community. Plant productivity does develop over time, but can still take decades to become equivalent to natural wetlands (Ballantine & Schneider, 2009; Hossler et al., 2011; Hossler & Bouchard, 2010; Moreno-Mateos et al., 2012). In contrast, created wetlands have been shown to have plant communities similar to natural wetlands in regards to composition, diversity and richness (Hossler et al., 2011). In terms of habitat, it is expected that this aspect will be similar between created and natural wetlands.

In addition to plants, macroinvertebrates tend to be highly affected by the water and soil characteristics of the site (Angradi et al., 2001; Brraich & Kaur, 2017). Hydrology, for example, is an important factor in community composition and individual success, as many macroinvertebrates require standing water to complete their entire life stage. Created wetlands can have more extreme hydrological regimes, often being exceptionally wet or very dry, while natural wetlands tend to be more stable and variable (Cole & Brooks, 2000; Hossler et al, 2011). The more unpredictable hydrologic environment observed in created wetlands could result in an even less established macroinvertebrate community, specifically if the wetlands are intermittent and not inundated year round. Hydrology is also an important factor in soil chemistry itself, which could mean it has a compounding impact on macroinvertebrates as a whole (Fennessy & Mitsch, 2001). Like plant communities, soils can be a food source via detritus, or critical habitat for many larval stages of macroinvertebrates. The soils of created wetlands are often deficient in carbon and nutrients, as these attributes require a long time to develop naturally (Hossler et al. 2011, Ballantine et al. 2009), which could mean that there is less available organic material for macroinvertebrates to utilize.

Although many studies have evaluated general macroinvertebrate community metrics (e.g. diversity, richness, relative abundance) in created and/or rehabilitated wetlands alone or in comparison to natural wetlands (Balcombe et al., 2005; Campbell et al., 2002; Hartzell, Bidwell, & Davis, 2007; Marchetti et al., 2010; Ruhí et al., 2013; Sartori et al., 2015; Spadafora et al., 2016; Thiere et al., 2009), none of these studies completed thorough trophic analyses. Trophic structure provides an understanding of how macroinvertebrates are organized rather than simply measuring how many macroinvertebrates are there. As such, trophic structure can provide more insight into the functions provided by macroinvertebrates, which is a major facet of services provided by wetlands and an endpoint for wetland mitigation. It is also suggested that trophic metrics of communities link biodiversity and ecosystem function (Thompson et al., 2012). In the progression from newly formed to mature systems, food-chain length and trophic complexity—two components of trophic structure—are expected to increase as the system becomes more productive (Odum, 1969). In addition to the initial increase in productivity, the successional changes in food-chain length and trophic complexity are expected to occur also because of increasing taxonomic diversity, habitat heterogeneity and food resource heterogeneity (Brown & Southwood, 1983; Neutel et al., 2007; Odum, 1969).

*The objective of this research* is to further the understanding of benthic macroinvertebrate community composition and trophic structure in created and natural wetlands. This knowledge will enable those involved with mitigation procedures to make more informed decisions particularly with respect to preservation of functions that might be mediated by macroinvertebrates. For five created and two natural marshes in central

and southwest Ohio, USA, I tested the following *central hypotheses*: (1) that benthic macroinvertebrate community composition in created wetlands will be statistically different from natural wetland community composition; and (2) that the trophic structure of benthic macroinvertebrate communities in created wetlands will be less complex and have a shorter food chain length than in natural wetlands. The first hypothesis follows from Ruhí et al. (2012) who showed that pioneer communities in created wetlands consist predominantly of active dispersers, and slowly approach community compositions similar to natural wetlands. The second hypothesis is based on Odum's (1969) theory of ecosystem development which suggests that newer systems will have lower primary production (at least initially) and therefore fewer resources to support a larger food-web.

## II. RESEARCH METHODS

### DESIGN AND RESEARCH APPROACH

The selected sites are all freshwater marshes that are palustrine emergent according to Cowardin (1992), and depressional according to Brinson (1993). There are five created wetland sites that fall between five to fourteen years past creation. There are two reference or natural wetlands, which are neither constructed nor remediated at any time. Of the two natural wetlands, Calamus Swamp is considered higher quality, more pristine and is rated category 3 according to the Ohio Rapid Assessment Method (ORAM) for wetlands; Dunlap is considered lower quality, more impacted and is rated category 2 by the ORAM. Site locations are provided in Table 1. Note, that two older created wetlands were additionally sample for stable-isotope analysis (SIA) only (Table 1). The range of created wetland age allowed me to see the trajectory of macroinvertebrate community succession within created wetlands and compare that progress to natural wetlands that will be used as reference.

### VEGETATION SURVEY

Vegetation surveys were completed in the summer of 2017. Baselines were created along the longest edge of each wetland, lengths were recorded, and the baselines were marked at 0.1, 0.3, 0.5, 0.7, and 0.9 of total baseline length. Transects were run along these divisions perpendicular to the baseline to the opposite edge of the wetland, lengths were recorded, and vegetation was surveyed at regular intervals of  $0.05 \times$  length of the transect. Vegetation species, percent cover, percent water coverage, percent open

water, water depth, and coordinates were recorded within a 0.25 m × 0.25 m quadrat frame at each of these points (Hossler & Bouchard, 2010).

Table 1: Created and natural wetland sites. \*Due to limited resources these sites were only used for SIA

Name	Age	Latitude	Longitude	Total Area (m <sup>2</sup> )	Sample Area (m <sup>2</sup> )
Glacier Ridge	5 yrs	40°7' 26.50"N	83°10' 58.14"W	1,626	930
Morgan Run	5 yrs	39°57'45.31"N	83°13'05.96"W	68,666	18,457
Champaign County	7 yrs	40°13'02.70"N	84°00'06.50"W	43,745	24,246
Preble Historical Society	7 yrs	39°39'33.74"N	84°32'50.42"W	10,830	5,355
Prairie Oaks	14 yrs	39°59'27.38"N	83°15'27.71"W	15,119	10,929
Larch Tree*	60 yrs	39°47'01.00"N	83°20'21.00"W	6,180	4,891
Possum Creek*	60 yrs	39°42'42.01"N	84°15'48.30"W	9,307	7,964
Dunlap	Natural	39°50' 03.30"N	82°43' 46.92"W	43,465	30,604
Calamus Swamp	Natural	39°35'03.33"N	83°00'01.58"W	69,228	34,252

## SAMPLING AREAS

Sampling areas were determined using the vegetation and hydrological data collected during the surveys, and quadrats not suitable for sampling were excluded from the randomized selection. Specifically, I excluded the quadrats that did not have enough standing water to adequately sample macroinvertebrates. It is important to make this distinction to optimize macroinvertebrate detection by removing areas in which presence is unlikely (e.g. infrequently inundated or completely dry areas). Vegetation community data was used to create strata within each wetland to ensure randomized sampling

effectively represented all microhabitats within each wetland. One quadrat was randomly selected from each strata for sampling of macroinvertebrates and soil.

### MACROINVERTEBRATES

Macroinvertebrate sampling was done by D-net sweeping twice within the sampling area. The first collection was to collect live organisms for stable isotope analysis (SIA), and was done by sweeping the net three times within 1 m<sup>2</sup> area by tapping the net against the soil three times followed by sweeping the open water to collect macroinvertebrates. Organisms had to be kept live and in site water to ensure the tissues remained intact for analysis. The second collection took place within 5 m of the quadrat location, but not in the exact location to avoid sampling an area that has been depleted of organisms. These samples were preserved in 70% alcohol to be used for general community metrics. All samples, live and preserved, were sorted to family and then counted in the lab using a dissecting microscope. Live samples were then frozen in glass vials, freeze dried, ground into homogenous powders, and packed into tins to be sent away to Washington State University for SIA which provided  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Currin et al., 2011) within macroinvertebrate tissues. It was necessary to combine some samples into larger groups, such as order, to ensure that there was an adequate sample for processing. The identified taxa were also assigned to functional feeding groups (FFG) following Barbour et al. (1999) and Merritt et al. (2008).

### SOIL SAMPLES

In addition to macroinvertebrate sampling, soil samples (10 cm diameter  $\times$  10 cm length) were taken at each sampling area. Bulk density was determined by the core method (Blake 1965). Particle-size distribution (sand, 50–2000  $\mu\text{m}$ ; silt, 2–50  $\mu\text{m}$ ; and

clay < 2  $\mu$  m) was determined by a modified pipette method (Gee & Or 2002, Gavlak et al. 2003). Subsamples of dried, ground, and homogenized soils were also packaged into tins and sent to Washington State University for C and N analysis.

## TROPHIC ANALYSES

Food-chain length (FCL) was determined by first establishing a baseline for each site, which was done by averaging  $\delta^{15}\text{N}$  signatures from all primary consumers within the sites, as these can be considered proxies for basal resources (Hoeinghaus et al., 2008; Walters et al, 2008; Hayden et al, 2016). I then calculated trophic position (TP) for each consumer group (i.e. family taxon) within each wetland as  $\text{TP} = [(\delta_c - \delta_b) / \Delta_n] + 2$ , where  $\delta_c$  is the  $\delta^{15}\text{N}$  signature of the consumer for which TP is to be estimated;  $\delta_b$  is the mean primary consumer  $\delta^{15}\text{N}$  signature (i.e.  $\delta^{15}\text{N}$  baseline) for the wetland where the consumer was sampled; and  $\Delta_n$  is the enrichment in  $\delta^{15}\text{N}$  per trophic level (i.e., 3.4 ‰) (Post, 2002, Anderson & Cabana, (2007)). I defined FCL to be the maximum TP within each wetland (Vander Zanden et al. 1999; Post et al. 2000). I then calculated range, minimum, mean and maximum for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  per wetland across all consumer groups. Finally, I used the used the SIAR package in R to estimate four additional metrics describing macroinvertebrate trophic structure:  $\delta^{13}\text{C}$  range (C13R); total convex hull area (TA); mean distance to centroid (CD); and mean nearest neighbor distance (MNND). These metrics followed Layman et al. (2007) and were calculated from the  $\delta^{13}\text{C}$ –  $\delta^{15}\text{N}$  bi-plot space per wetland.

## STATISTICAL ANALYSIS

I first tested for effects of type (i.e. created or natural wetland) and age (created wetlands only). For univariate data, I used analysis of variance (ANOVA) to test for type



effects and simple linear regression to test for age effects. It is important to note that due to the high number of univariate testing done on the data, False Discovery Rate (FDR) was used to control for multiple hypothesis testing (Benjamini and Hochberg 1995). For multivariate data, I assessed effects visually through either nonmetric multidimensional scaling (NMDS) or principal component analysis (PCA), and formally through permutational multivariate analysis (PERMANOVA). Specifically, NMDS (with Jaccard distances) was done for taxa, FFG, and vegetation compositions to visually show the relationships between communities. PCAs were done with soil and trophic metrics to visually assess the magnitude of variance explained by the variables. The trophic-based metrics included food-chain length,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ranges, and trophic area as an indicator of trophic diversity (see Layman et al. 2007). NMDS and PCA were performed on station-level data (i.e.  $n = 3$  per site), while PERMANOVA and the univariate analyses were performed on weighted means (i.e.  $n = 1$  per site; weighted means were calculated using strata weights from the vegetation surveys).

I then tested for effects of the three primary wetland structural components (i.e. vegetation, soil, hydrology) on the macroinvertebrate communities: first individually through canonical correspondence analysis (CCA); then collectively through variation partitioning. While CCA shows the relationships between the communities and environmental factors individually, variation partitioning shows the variation explained by each habitat characteristic individually and variation explained by the characteristics collectively.

Because size is a known driver of many community metrics (e.g. diversity, FCL; Post et al. 2000), I tested whether total wetland area or sampled wetland area (Table 1)

biased any observed relationships. Although sampled area (but not total area) was significantly higher in natural wetlands than in created wetlands, I did not detect an undue influence of size by simple regression (i.e. parameter versus size) or model selection (i.e. model comparisons with and without size as a cofactor).

All calculations and statistical analyses were performed in R 3.1.2 (R Core Team 2014). Diversity metrics and multivariate analyses (i.e. NMDS, PCA, CCA, PERMANOVA, variation partitioning) utilized the R package VEGAN (Oksanen et al., 2015). Specifically, the VEGAN functions *metaMDS*, *rda*, *cca*, *adonis* and *varpart* were used for NMDS, PCA, CCA, PERMANOVA and variation partitioning. Additionally as noted, the R package SIAR (Parnell & Jackson, 2013) was used to estimate C13R, TA, MNND, and CD.

### III. RESULTS

#### MACROINVERTEBRATE COMMUNITY COMPOSITION

In total, 10,179 macroinvertebrates were collected. In created wetlands, Chironomidae (midges), Physidae, and Valvatidae (both types of snail) were the most abundant taxa, while Caecidotea (isopods), Pleidae (small predatory beetle), and Culicidae (mosquito), were the most abundant in natural wetlands. There was no statistical difference in macroinvertebrate diversity when comparing wetland type or created wetland age (Fig. 1; Table 2). In addition, wetland types were not significantly different regarding macroinvertebrate richness or functional feeding group diversity, there was also no significance when looking at age (Fig. 2, Fig. 3). Macroinvertebrate community composition, however, was statistically different between wetland types (PERMANOVA:  $F_{1,5} = 1.506$   $p = 0.05$ ; Fig. 4), and there was a statistical trend between wetland types for macroinvertebrate FFG composition (PERMANOVA:  $F_{1,5} = 1.861$ ,  $p = 0.095$ ; Fig. 5). Most notably, the proportion of predators was lower in created wetlands than in the natural wetlands (Fig. 5): a relationship which was further supported by univariate regression ( $p = 0.011$ ; Table 2). Univariate regression also suggested a trend between predator proportion and created wetland age ( $p = 0.015$ ; Table 2), although there was no statistical relationship with created wetland age for either macroinvertebrate community or macroinvertebrate FFG composition (all  $p > 0.1$ ; Fig. 4, Fig. 5). Proportions of GC, FC and SH (i.e. detritivores), considered individually and combined, were statistically similar between created and natural wetlands ( $F_{1,5} = 1.59$ ,  $p = 0.263$ ).

When looking at the detritivores with age, there was a significant trend of a decrease with age ( $F_{1,3} = 17.3$ ,  $p = 0.025$ ; Table 2). Interestingly, when I regrouped the wetlands into young-created (GR, MR, CC, and PHS) and older-created and natural (PO, CAL, and DUN), differences by “type” became significant ( $F_{1,5} = 20.1$ ,  $p = 0.0065$ ), with higher proportions of detritivores in the young-created wetlands.

### MACROINVERTEBRATE TROPHIC STRUCTURE

To improve sample size in my analysis of macroinvertebrate trophic structure, two sixty-year-old created wetlands were added to this portion of the study: Possum Creek and Larch Tree. Mean macroinvertebrate  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ranged from 3.31 ‰ to 6.8 ‰ and -32.8 ‰ to -23.1 ‰, respectively, and were not significantly different between wetland types or across created wetland age. Estimates of mean trophic position (TP; Fig. 6) and food chain length (FCL; Fig. 7) were not statistically different based on wetland type (mean TP,  $p = 0.084$ ; FCL,  $p = 0.462$ ) or created wetland age (mean TP,  $R^2 = 0.066$ ,  $p = 0.578$ ; FCL,  $R^2 = 0.389$ ,  $p = 0.134$ ). When looking at the remaining SIA-based metrics (total area (TA), centroid distance (CD), mean nearest neighbor distance (MNND), mean  $\delta^{13}\text{C}$ , and mean  $\delta^{15}\text{N}$ ), there were no significant differences between created and natural wetlands or with age of created wetlands. A PCA comparing the overall trophic structure further suggested no significant difference between wetland types or with created wetland age (Fig. 8).

Table 2: Summary of statistical results for all variables analyzed against wetland type and age of created wetlands. Sample size was 7 for all tests except the SIA-based metrics (e.g. FCL, Mean TP), which had a sample size of 9. Significant values in bold and trends in bold italic. Significance threshold of  $\alpha = 0.05$  and trend threshold of  $\alpha = 0.1$ ; thresholds were adjusted by FDR to control for multiple hypothesis testing. Detritivores defined as shredders (SH), gatherer/collectors (GC), and filterer/collectors (FC). Effect sizes were calculated for the type-based ANOVAs ( $d$ ) and age-based regressions ( $f^2$ ) per Cohen (1988).

Variable	Type			Age			
	F	$p$	Cohen's $d$	F	$R^2$	$p$	Cohen's $f^2$
Macro Richness	0.28	0.615	0.449	1.914	0.390	0.260	0.638
Macro Diversity	4.70	0.082	1.814	2.299	0.434	0.227	0.766
FFG Diversity	0.11	0.748	0.284	0.685	0.186	0.469	0.228
GC	0.630	0.464	0.597	3.711	0.553	0.150	1.156
SC	0.515	0.505	0.635	8.568	0.741	0.061	2.235
PR	<b>15.36</b>	<b>0.011</b>	<b>2.293</b>	<b>25.216</b>	<b>0.894</b>	<b>0.015</b>	<b>8.405</b>
SH	0.74	0.428	0.769	0.132	0.042	0.741	0.076
FC	0.933	0.378	0.837	0.314	0.095	0.615	0.037
Detritivores	1.59	0.263	1.055	<b>17.309</b>	<b>0.852</b>	<b>0.025</b>	<b>5.770</b>
FCL	0.60	0.462	0.623	3.193	0.39	0.134	0.638
Mean TP	3.88	0.089	1.579	0.353	0.066	0.578	0.070
TA	0.12	0.735	0.282	3.258	0.395	0.131	0.651
CD	0.86	0.385	0.743	3.566	0.416	0.118	0.713
MNND	0.25	0.629	0.405	4.869	0.493	0.078	0.973
C13R	0.56	0.478	0.600	1.276	0.203	0.310	0.255
Mean13C	0.00	0.927	0.076	0.001	0.00	0.975	0.0002
Mean15N	2.01	0.199	1.137	0.015	0.003	0.906	0.003
Veg Richness	0.02	0.875	0.139	0.005	0.002	0.950	0.002
Veg Diversity	0.03	0.852	0.165	0.545	0.154	0.514	0.182
Mean Water Depth	0.03	0.870	0.144	2.818	0.484	0.192	0.939
Soil, BD	<b>14.81</b>	<b>0.012</b>	<b>3.220</b>	2.158	0.418	0.238	0.719
Soil, GW	4.372	0.091	1.754	4.585	0.605	0.122	1.348
Soil, OM	8.13	0.036	2.386	0.112	0.036	0.759	0.236
Soil, pH	3.16	0.135	1.496	0.160	0.051	0.716	0.012
Sand	3.503	0.120	1.371	0.068	0.022	0.812	0.027
Silt	<b>21.68</b>	<b>0.006</b>	<b>3.896</b>	0.693	0.188	0.466	0.178
Clay	0.025	0.881	0.259	0.479	0.138	0.539	0.123

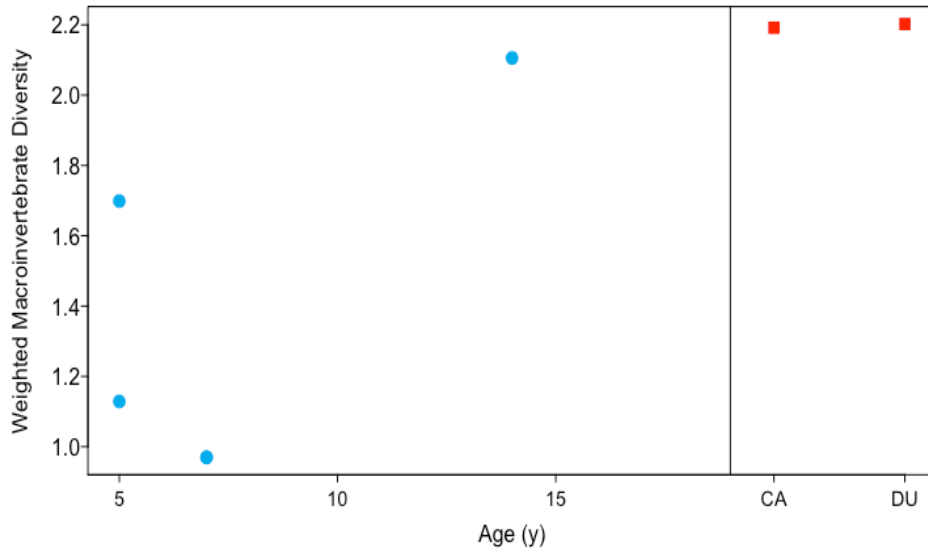


Figure 1: Macroinvertebrate diversity was not impacted significantly by wetland type ( $F= 4.703$ ,  $p= 0.082$ ) or age ( $F= 2.299$ ,  $R^2= 0.434$ ,  $p= 0.227$ ). (Blue circles = created wetlands; red squares = natural wetlands)

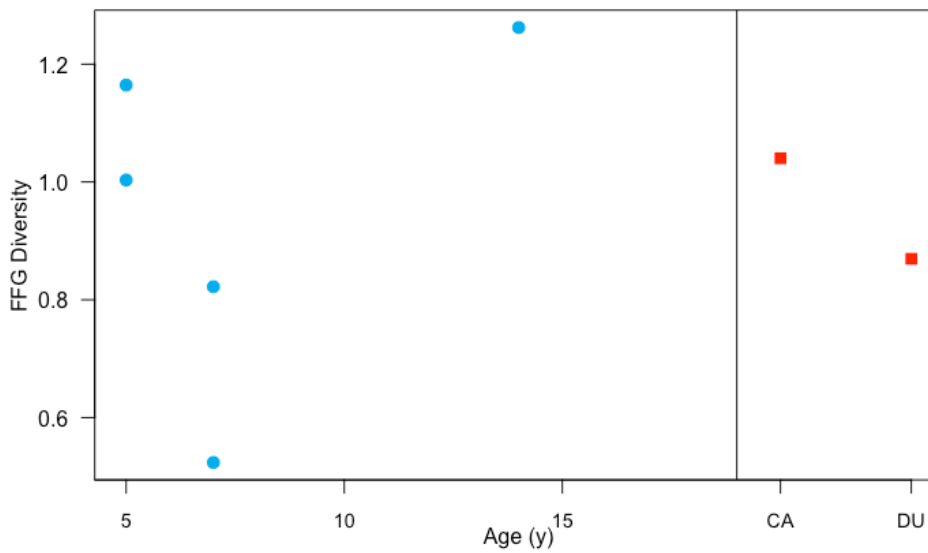


Figure 2: FFG diversity was not impacted significantly by wetland type ( $F= 0.115$ ,  $p= 0.748$ ) or age ( $F= 0.685$ ,  $R^2= 0.186$ ,  $p= 0.496$ , see also Table 2). (Blue circles = created wetlands; red squares = natural wetlands)

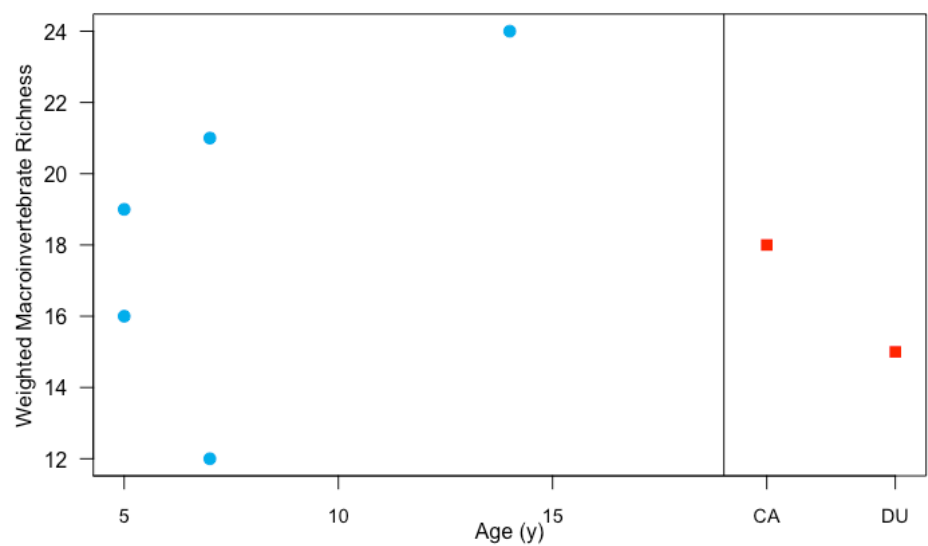


Figure 3: Macroinvertebrate richness was not impacted significantly by wetland type ( $F= 0.287$ ,  $p= 0.615$ ) or age ( $F= 1.914$ ,  $R^2= 0.390$ ,  $p= 0.260$ , see also Table 2). (Blue circles = created wetlands; red squares = natural wetlands)

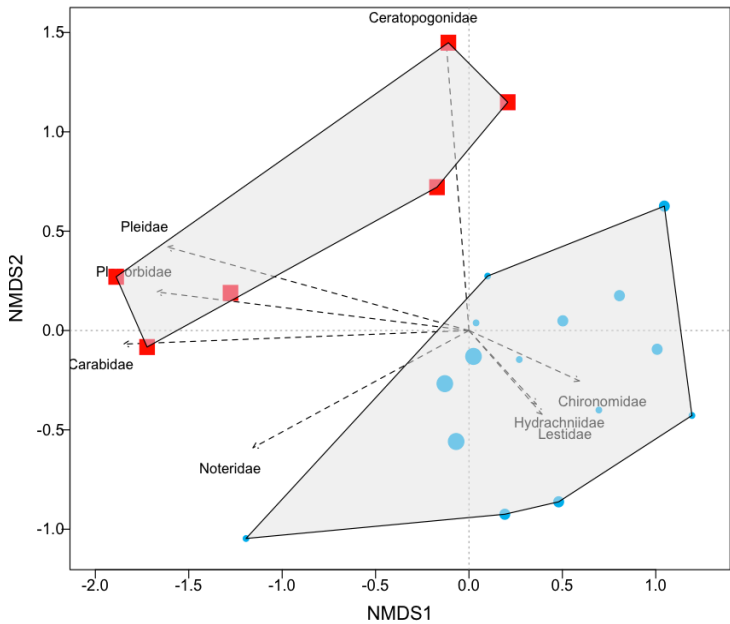


Figure 4: NMDS showing the difference between macroinvertebrate community taxa composition between natural and created wetlands ( $F_{1,5} = 1.506, p = 0.05$ ), and age ( $F_{1,3} = 0.878, p = 0.733$ ). Note that the NMDS used station-based data ( $n = 21$ ), while the PERMANOVA used weighted means ( $n = 7$ ). (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

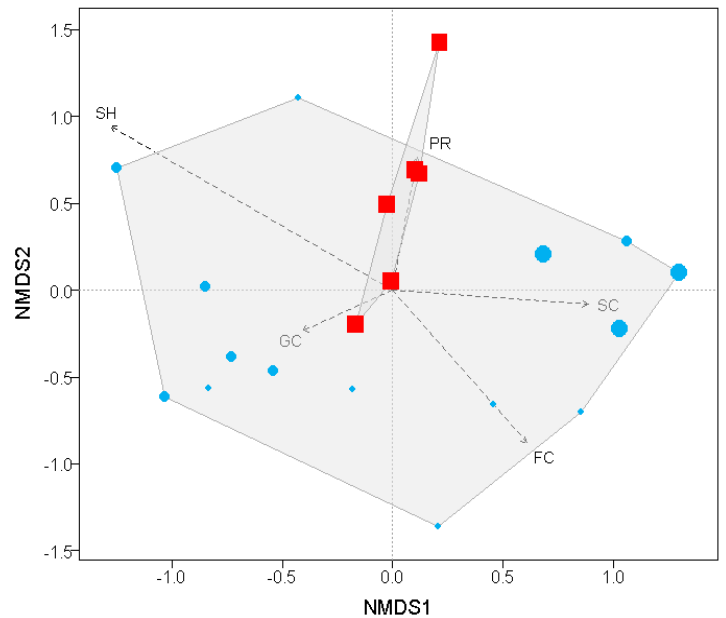


Figure 5: NMDS showing the difference between FFG composition between natural and created wetlands ( $F_{1,5} = 1.861, p = 0.095$ ) and age ( $F_{1,3} = 0.979, p = 0.533$ ). Note that the NMDS used station-based data ( $n = 21$ ), while the PERMANOVA used weighted means ( $n = 7$ ). (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

## MACROINVERTEBRATE HABITAT CHARACTERISTICS

I then compared plant, soil and hydrologic habitat characteristics expected to impact macroinvertebrate community composition and trophic structure. Vegetation diversity, richness and composition were similar between created and natural wetlands and exhibited no statistical relationships with created wetland age (all  $p > 0.1$ ; see Fig. 9 for the composition NMDS). CCA suggested a significant relationship between macroinvertebrate and vegetation communities ( $F_{12,8} = 1.45, p = 0.013$ ; Fig. 10), but not between FFG and vegetation communities ( $F_{12,8} = 1.10, p = 0.47$ ; Fig. 11).

In contrast to vegetation, created and natural wetlands were quite distinct in terms of soil properties (PERMANOVA:  $F_{1,5} = 4.65, p = 0.043$ ). PCA suggested that soil OM, GW and silt were lower and soil BD was higher in created wetlands as compared to

natural wetlands (Fig. 12). This was supported by univariate regressions of soil properties by wetland type, which found significant differences when looking at soil BD ( $p = 0.012$ ) and silt ( $p = 0.006$ ; Table 2). There were no significant differences or trends when looking at the impact of age on soil properties in created wetlands (Table 2). When comparing macroinvertebrate communities and soil properties using CCA, there was a significant relationship for family-level composition ( $F_{6,14} = 1.79$ ,  $p = 0.003$ ; Fig. 13) and a trend for FFG composition ( $F_{6,14} = 1.71$ ,  $p = 0.068$ ; Fig. 14). Specifically, BD, pH, and silt are trending with younger created wetlands as well as the natural wetlands. Increasing BD and pH are trending with an increase in detritivore presence while increasing silt is trending with an increase of predators

Hydrology had one of the weakest correlations to macroinvertebrate communities, with water depth having non-significant relationships to family-level taxa and FFG composition ( $F_{1,19} = 1.27$ ,  $p = 0.102$  and  $F_{1,19} = 1.54$ ,  $p = 0.172$ , respectively). There was no statistical relationship between water depth and wetland type ( $p = 0.870$ ) or created wetland age ( $p = 0.192$ ; Table 2).

Through variation partitioning, I show the individual and collective impact that habitat characteristics have on macroinvertebrate communities with an overall percentage of variation explained at 0.77 (Fig. 15). Of the three primary wetland structural components, soil alone accounted for the most variation (0.61), followed closely by vegetation (0.53). Hydrology accounted for only 0.20 of the variation in macroinvertebrate communities, on par with the combination of all habitat characteristics at 0.25. All other combinations of characteristics were less than zero. Variation partitioning using FFG showed similar breakdown.



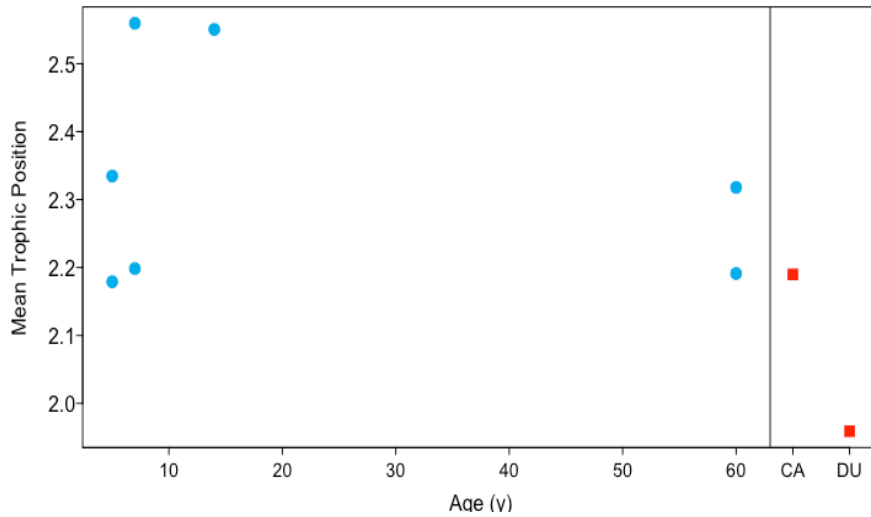


Figure 6: Mean trophic position is not impacted significantly by wetland type ( $F= 4.703, p= 0.082$ ) and age ( $F= 2.299, R^2= 0.434, p= 0.227$ ). (Blue circles = created wetlands; red squares = natural wetlands)

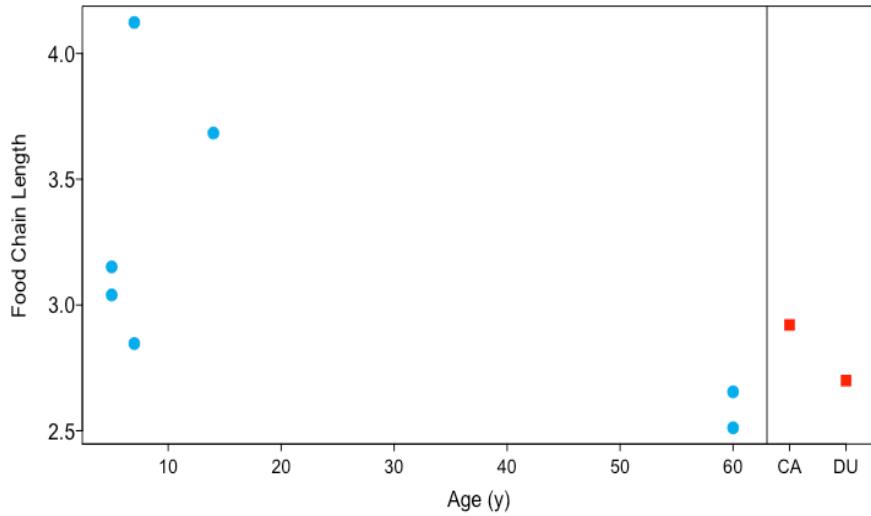


Figure 7: Macroinvertebrate food chain length is not impacted significantly by wetland type ( $F= 0.287, p= 0.615$ ) and age ( $F= 1.914, R^2= 0.390, p= 0.260$ ). (Blue circles = created wetlands; red squares = natural wetlands)

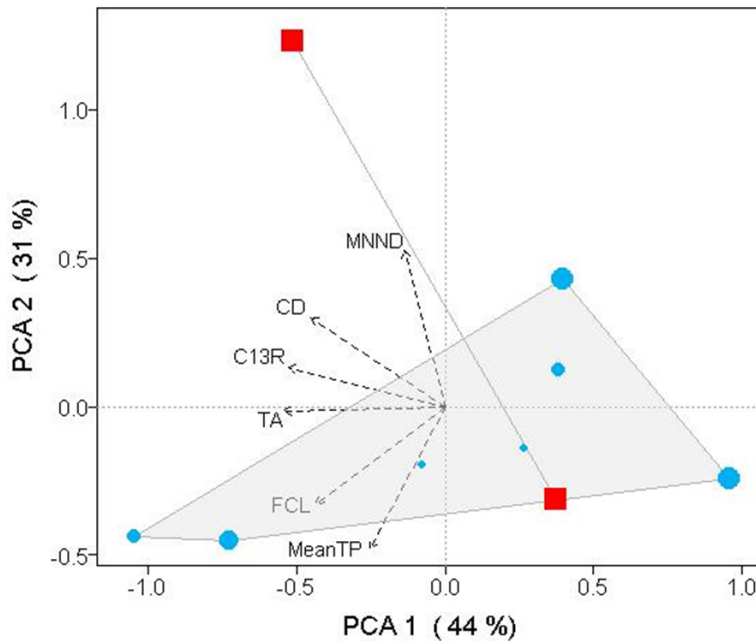


Figure 8: PCA showing how SIA metrics correlate to wetland type ( $F_{1,7}= 0.528, p= 0.785$ ), and age ( $F_{1,5}= 2.181, p= 0.128$ ). 2D variance is 0.693. (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

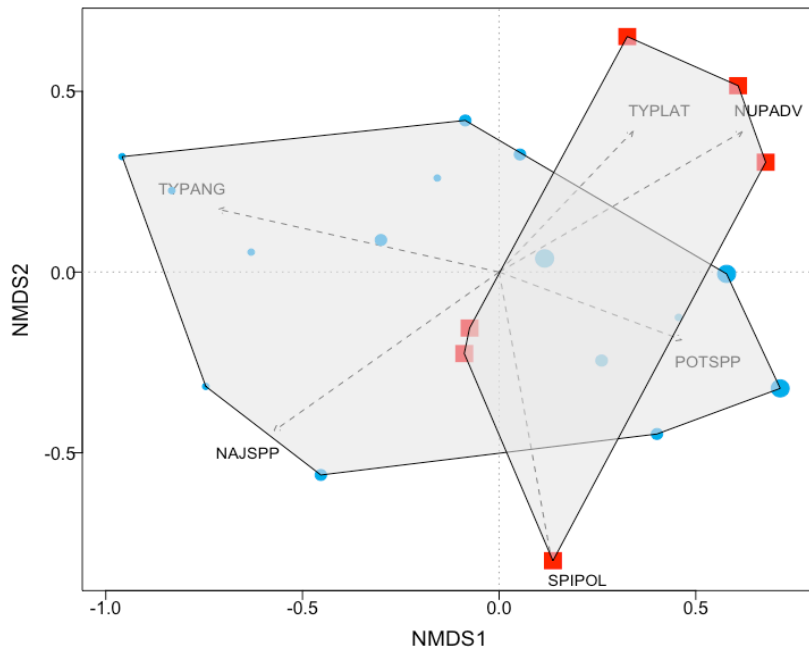


Figure 9: NMDS showing no significant differences in wetland vegetation with type, ( $F_{1,5}=1.029$ ,  $p=0.32$ ) or age ( $F_{1,3}=0.875$ ,  $p=0.8$ ) with stress at 0.14. Note that the NMDS used station-based data ( $n=21$ ), while the PERMANOVA used weighted means ( $n=7$ ). (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

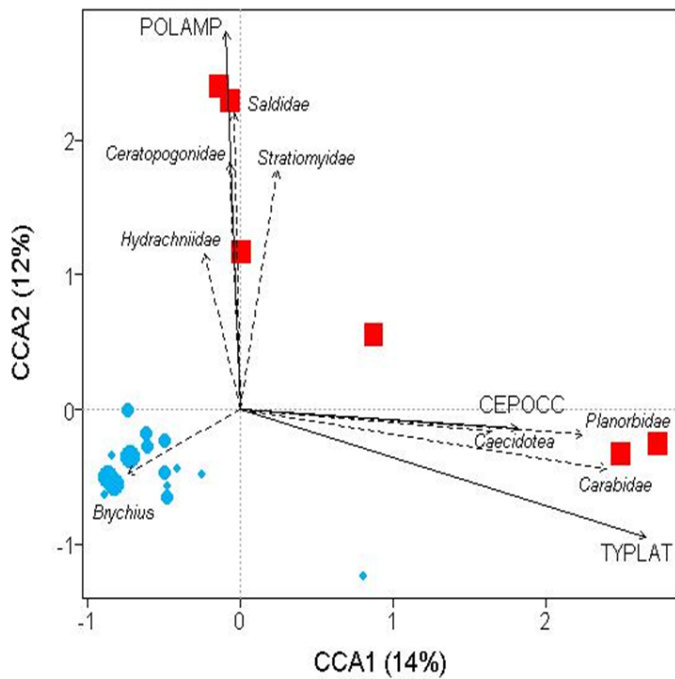


Figure 10: CCA comparing macroinvertebrate taxa composition and vegetation composition. 2D variance 0.267, total variance= 0.685,  $p=0.013$ ,  $F_{12,8}=1.4504$  (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

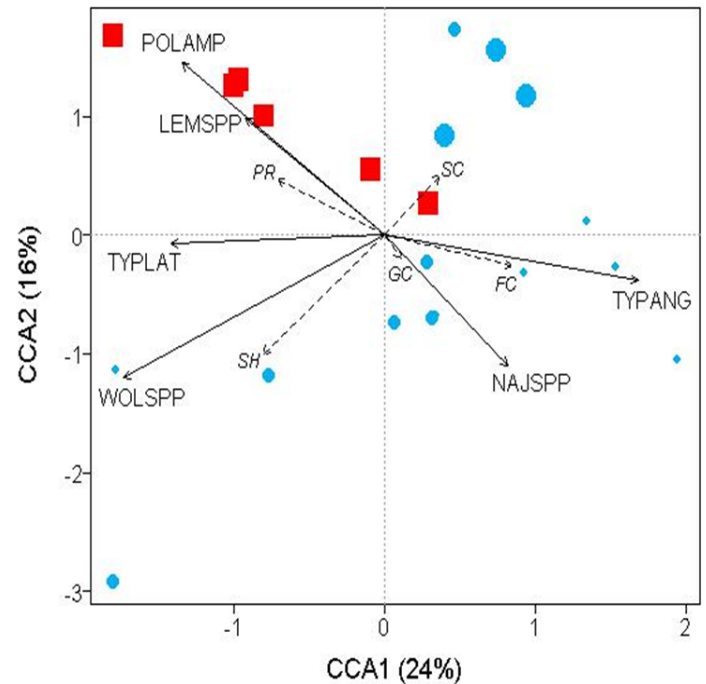


Figure 11: CCA showing the correlation between vegetation community composition and FFG proportions. 2D variation= 0.404, total variance= 0.601,  $p=0.47$ ,  $F_{12,8}=1.0025$  (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

#### IV. DISCUSSION

This study examined created and natural wetland sites with the goal of determining how created wetlands differ in macroinvertebrate composition, trophic structure, and habitat characteristics. In addition, the ages of created wetlands were considered to understand if there was any trajectory with age. While macroinvertebrate diversity and richness were not significantly different, overall community compositions were different between wetland types. I expected differences in trophic structure, however created and natural wetlands appeared very similar across a variety of trophic-related metrics. With respect to habitat characteristics, vegetation and hydrology were similar as anticipated, but there were substantial differences in soil between wetland types. Namely, created wetland soils were significantly more dense and less silty than natural wetland soils. With respect to macroinvertebrate community composition, the soil habitat appeared most important, followed by vegetation.

Perhaps the most interesting finding of this study is regarding the macroinvertebrate communities. While the richness and diversity of communities was similar across all wetland sites, both the taxa and FFG compositions of natural wetlands were statistically different when compared to communities in created wetlands. It is important to note that there was no trend with age for community composition. These findings do reflect some previous studies. Balcombe et al. (2005) reported that created and reference wetlands had equally abundant, diverse, and productive macroinvertebrate communities with only three taxa being statistically different between wetland types.

Spadafora et al. (2016) also reported that the macroinvertebrate communities in their wetlands differed in composition between wetland types. However, they also reported that the macroinvertebrate community of the studied created wetland had higher diversity than the reference (natural) wetland. A meta-analysis by Moreno-Mateos et al. (2012) (161 data points) found that macroinvertebrate density in restored and constructed wetlands took between five and ten years to converge with reference populations. The results presented in this paper do not reflect that finding, as even the younger wetland sites have similar community metrics to the natural wetlands. Collectively, my study and previous studies suggest that from a numbers perspective (i.e. diversity and richness) created wetlands are similar to natural wetlands—or become so within a fairly short time frame—, although compositionally (i.e. which species) they differ.

More important is the understanding of macroinvertebrate functionality in created wetlands. To my knowledge, no previous study has compared macroinvertebrate functionality or trophic structure in created and natural wetlands, and this was the main objective of my research. Trophic structure is an essential link between biodiversity and ecosystem function (Thompson et al., 2012), which is why I first compared FFG composition. This comparison suggested a trend towards significance in macroinvertebrate function between wetland types. However, macroinvertebrate communities were much more distinct between created and natural wetlands when evaluating family-level taxa. This implies that while the species present differ, the created wetlands may be functioning similarly to natural wetlands. Using the gatherer-collector FFG as an example, the natural wetlands had a higher abundance of Culicidae, while created wetlands had higher Chironomidae.

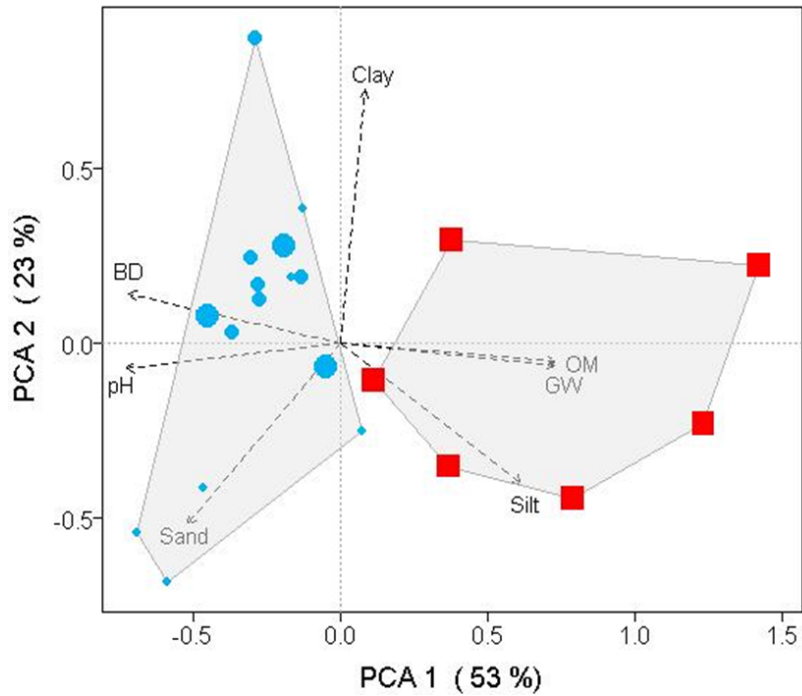


Figure 12: PCA depicting station data shows the significant difference between soil characteristics of natural and created wetlands ( $F_{1,5} = 4.649, p = 0.043$ ) but no significance with age ( $F_{1,3} = 0.552, p = 0.667$ ). (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

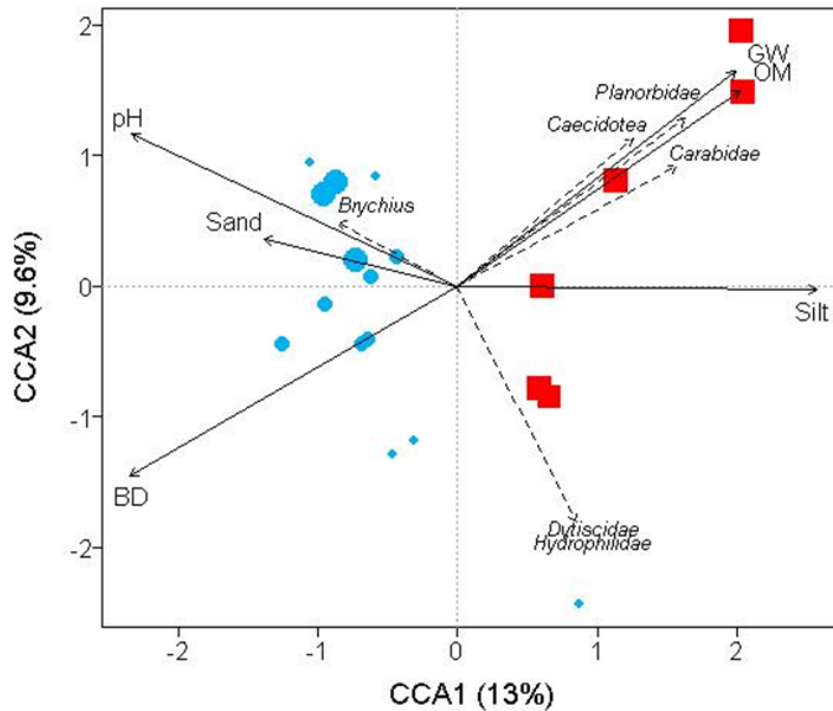


Figure 13: CCA comparing macroinvertebrate taxa and soil properties. 2D variation = 0.229, total variance = 0.433,  $p = 0.003, F_{6,14} = 1.7852$ . (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

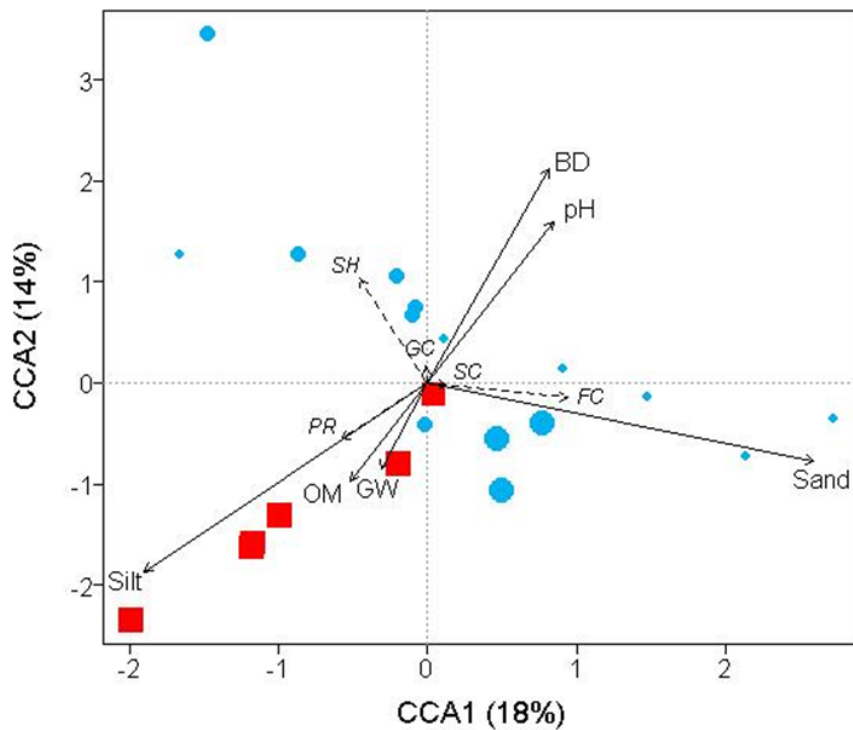


Figure 14: CCA comparing FFG composition and soil characteristics. 2D variation= 0.323, total variation= 0.423,  $p= 0.068$ ,  $F_{6,14}= 1.7104$ . (Blue circles = created wetlands sized to represent age, larger circles are older wetlands; red squares = natural wetlands)

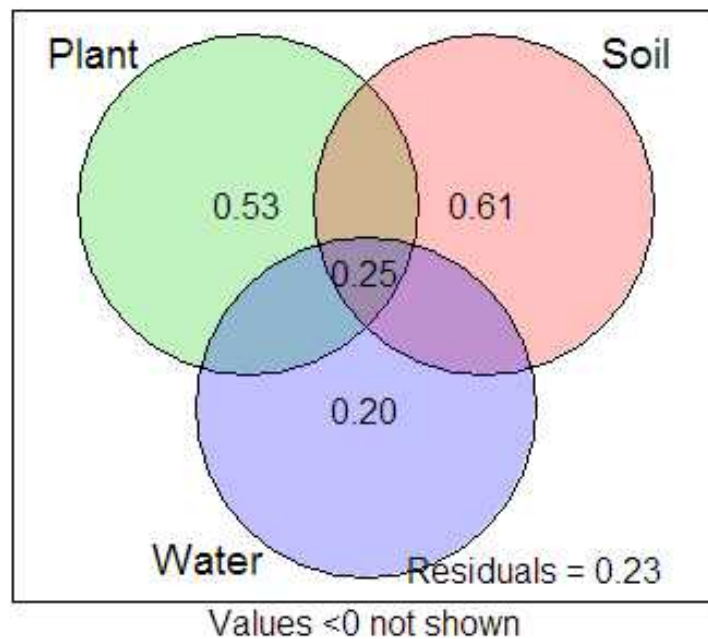


Figure 15: Diagram showing the results of variance partitioning of macroinvertebrate data. The three explanatory data frames were vegetation communities ("Plant"), soil characteristics ("Soil"), and hydrology ("Water"). The values shown represent the portion of variation accounted for by that explanatory data frame.

Understanding not only which organisms are present in a community, but their functional roles, can provide important insight into valued ecosystem services such as decomposition and nutrient cycling. One unexpected result was the relative abundance of detritivores—i.e. the gatherer/collectors (GC), filterer/collectors (FC) and shredders (SH) (Cummins et al. 1973). While this grouping of detritivores does not distinguish between shredding-herbivores and shredding-detritivores, it is the most accurate grouping for these analyses (Anderson & Sedell, 1979). Although the initial detritivore analysis suggested no difference relative proportions between created and natural wetlands, there was a significant decreasing trend with created wetland age and statistically significant difference when regrouped into young-created (GR, MR, CC, and PHS) and older-created and natural (PO, CAL, and DUN) wetlands. Freshwater wetlands are often considered detrital-based systems (Brinson et al. 1981, Williams & Trexler 2006, Spieles & Mora 2007; Batzer et al. 2014) and I expected that detrital-reliance would be less important in created wetlands based on studies documenting lower amounts of litter (e.g. Hossler et al. 2011) and slower rates of decomposition (e.g. Fennessy et al. 2008). The unexpected finding of larger proportion of detritivores in younger created wetlands could be due to the apparent increased terrestrial input in younger created sites. This can be assumed from the lower mean  $\delta^{15}\text{N}$  in younger created wetlands, which has been shown to correlate more to terrestrial vegetation (Reid, *et al.*, 2012). This relationship could also be due to the fact that the FFG proportions are relative and the relationship is being driven by the strong correlation of PR in older created and natural wetlands, with a higher proportion of predators in the natural wetlands in particular.

In contrast, there was no statistical difference in FCL between created and natural wetlands. In fact, my SIA-based analyses further suggested that trophic structure was similar between created and natural wetlands. This was contrary to my prediction that the natural wetlands would have a more complex trophic structure. In addition to FCL, for example, I observed similarities in range of  $\delta^{13}\text{C}$  (i.e. wider variety of basal resources; Layman et al. 2007) and overall total isotopic area (TA) between created and natural wetlands.

In regards to allochthonous vs. autochthonous carbon input in these systems, I expected that created wetlands would have a higher proportion of allochthonous carbon due to a lower rate of primary production (Hossler et al, 2011, Spieles D & Mora J, 2007) and therefore a larger reliance terrestrial input.  $\delta^{13}\text{C}$  is typically used to distinguish basal carbon sources, but can be problematic particularly in discriminating emergent aquatic vegetation from terrestrial vegetation (Bunn and Boon 1993, Benetti et al. 2014), and algal isotopic signatures can also be quite variable.  $\delta^{13}\text{C}$  signatures can also be impacted by land use, however the strongest drivers have been found to be DIC and pH (Chappuis *et al.*, 2017). There is also a large amount of overlap of  $\delta^{13}\text{C}$  ranges from photosynthetic pathways so it can be difficult to parse out the actual source of C within the samples. To account for this, a few studies have suggested that  $\delta^{15}\text{N}$  can distinguish allochthonous vs. autochthonous C, with  $\delta^{15}\text{N}$  signatures of terrestrial plants being more depleted than those of aquatic plants, such as algae and macrophytes (Fazekas & Vadeboncoeur, in prep), Sullivan et al. (Accepted), Benetti et al. 2014). Looking at  $\delta^{15}\text{N}$  for primary consumers, excluding one anomalous created wetland,  $\delta^{15}\text{N}$  was significantly lower (i.e. more terrestrial in origin) in the created wetlands ( $F_{1,6} = 20.13$ ,  $p = 0.004$  and  $F_{1,6} = 14.6$ ,  $p =$



0.009 for the mean and baseline  $\delta^{15}\text{N}$ , respectively) and increased with age ( $F_{1,4} = 22.6$ ,  $p = 0.009$  and  $F_{1,4} = 9.34$ ,  $p = 0.038$  for the mean and baseline  $\delta^{15}\text{N}$ , respectively). It is also necessary to consider other ways in which baseline  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  can be impacted through natural processes. Biologically driven reactions, such as denitrification, nitrification, and assimilation, strongly control nitrogen dynamics in the soil (Kendall *et al.* 1995; Kendall *et al.*, 1998). These reactions almost always result in  $^{15}\text{N}$  enrichment of the substrate and depletion of the product. For example, areas where water is more stagnant have lower  $\delta^{15}\text{N}$  values than well-drained soils (Karamanos *et al.*, 1981), perhaps because the greater denitrification in more boggy areas results in heavy residual nitrate. Other aspects such as land use and agricultural runoff can impact  $\delta^{15}\text{N}$  signatures drastically, with urban/agricultural areas having a less negative  $\delta^{15}\text{N}$  signature, and natural areas having a more negative  $\delta^{15}\text{N}$ . Aquatic plants are more susceptible to stronger  $\delta^{15}\text{N}$  inputs due to their varied sources and limited isotopic discrimination (Chappuis *et al.*, 2017; Peipoch *et al.*, 2012). In addition, nitrogen fixation discriminates against  $\delta^{15}\text{N}$  which can cause a buildup of  $^{15}\text{N}$  in systems where fixation is high.

It is likely that macroinvertebrates can quickly colonize the created wetland systems due to aerial life stages and rapid reproduction and life cycles (Stanczak, 2004, Balcombe, *et al.*, 2005). The resulting macroinvertebrate communities can develop in a way that emulates the natural wetland, particularly when the created wetland was constructed with the purpose of replicating a natural system. However, some macroinvertebrate taxa cannot easily colonize new habitats without the aid of other organisms or water as transportation (Moreno-Mateos, 2012; Levin & Talley, 2002; Figeula *et al.*, 2005).

I next evaluated primary habitat components (vegetation, soil, water) in terms of how they compared between created and natural wetlands and their importance in structuring macroinvertebrate communities. Regarding vegetation composition within all wetland sites, there were no statistical differences between types or any changes with age. The created wetlands used in this study were mostly found in city parks and were designed to emulate natural wetlands via the planting or seeding of plants commonly found in natural wetlands across Ohio. This is most likely the reason for the similarities between the vegetation communities. Vegetation-based metrics are frequently used to monitor wetland creation and mitigation projects (Van den Bosch & Matthew, 2017, Matthews & Endress, 2008) and other studies suggest that vegetation communities of created wetlands can develop rather quickly to resemble natural sites, even if not purposefully planted (Hossler *et al.*, 2011, Moore H *et al.* 2002, Matthews J *et al.* 2009).

Despite the similarities in created and natural wetland vegetation, vegetation was a significant driver of macroinvertebrate community composition. One reason may be the difference in spatial distribution between created and natural wetlands. For example, created wetland sites often consisted of tall emergent vegetation, such as *Typha* spp. or *Cyperaceae*, at a well-defined wetland perimeter with little-to-no transitional area between the wetland and surrounding landscape. The wetlands then usually had a steep slope as it progressed into standing water and dominating vegetation was submergent. Natural wetlands had more variable vegetation presence throughout the sites, with emergent vegetation being present in the center as well, likely due to larger wetland sizes and inconsistent water depth.

The most significant differences observed between created and natural wetlands are seen through looking at the soil characteristics. I expected to see most of the soil characteristics to be significantly different between wetland types, and possibly a trajectory with age that would begin to converge on natural wetland levels. While not every measured metric showed significance, both bulk density and percentage of silt were found to be significantly different between wetland types. The significance in bulk density is most likely due to the amount of time it takes for wetland soils to develop these characteristics, while other soil characteristics can develop more quickly (Hossler K & Bouchard V, 2010, Hossler K *et al.* 2011, Ballantine K & Schneider R, 2009). As bulk density and organic matter are typically closely linked, the lack of significance when looking at organic matter is surprising. While it is higher in natural sites, it does not reach the threshold to be considered significant or a trend, which does not match what I found regarding bulk density. This could be because OM has a greater variability than BD: the relationship between BD and OM is non-linear; as OM accumulates, BD decreases until it reaches a minimum threshold (the density of OM), while OM will continue to increase. Based on the OM and clay content of the soil, the created wetlands would all be considered mineral based. Dunlap is would also be considered mineral based, while Calamus Swamp is organic based (Soil Survey Staff 2014). These categories could be an underlying driver of macroinvertebrate communities shown via CCA. The correlations shown in the CCAs between soils and macroinvertebrate taxa (Fig. 13) and FFG (Fig. 14) are in-line with previous research (Ruggiero & Merchant, 1979). This is also echoed by the understanding we have of the importance of soil in macroinvertebrate distributions (Richards, 1993; Wright & Mattice, 1981). Similarity of soils between wetland types is

reflective of the similarity found in the vegetation communities. Soil is known to be a driver of vegetation diversity and the success of individual species in wetlands (Batzer and Wissinger, 1996), which is thought to have a correlated effect on macroinvertebrate assemblages as well (Angradi *et al.* 2001; Brraich *et al.*, 2017), visualized by the variance partitioning (Fig. 15).

It is important to not understate the role of hydrology in structuring macroinvertebrate communities. While the presence of standing water is required for the success of early life stages of certain macroinvertebrates, it is also known that macroinvertebrate community composition can strongly correlate to hydrological parameters such as water depth and inundation periodicity (Hugues *et al.*, 2008; Skoulikidis *et al.*, 2009; Tall *et al.*, 2015). Gleason and Rooney (2018) found that the hydrological factors driving community composition the most were drawdown date, maximum water depth, and the change in water depth during the season. Zimmer *et al.* (2000) described average water depth of wetlands as one of two secondary factors (primary factor in this study was presence of fathead minnows) that influenced macroinvertebrate composition, the other of these being vegetation abundance. While this study did not find a correlation between water depth and any macroinvertebrate metrics, this could be because water depth was sampled only one time (albeit multiple locations per wetland) during this study. Wetland depth can vary dramatically over a year in freshwater marshes (e.g. Mitsch & Gosselink). Hossler *et al.* (2011) monitored water depth in 10 created and 5 natural marshes of Ohio over one year, however, and found their hydrologic regimes (e.g. mean/minimum/maximum water depth, water depth variability, proportion of time inundated) to be similar.

## V. CONCLUSION

While the macroinvertebrate taxa composition in created wetlands differ from the natural sites, FFG composition only shows a trend towards significance. In addition, SIA-based trophic metrics, and taxa diversity and richness are similar between types, which implies that function of the macroinvertebrate communities may also be similar between wetland types. The observation that vegetation communities are similar between types is important in regards to the practice of creating wetlands for compensatory mitigation. It shows that vegetation communities in created wetlands are able to emulate their natural counterparts, while the soil characteristics are fairly distinct. Both vegetation and soil appear important in structuring macroinvertebrate communities and may be driving the observed differences in macroinvertebrate taxa composition. The only correlation found with age is in FFG composition, with more predators being found in the older created and natural sites, and a higher number of detritivores in younger created wetlands. The lack of further correlation between any measured variable with age suggests that the age range and sample size needs to be expanded in further research to parse out any possible impact age of created wetlands has on their similarity to natural wetlands. In particular soil properties such as bulk density and organic matter content can take very long to develop, which could hinder the ability of created wetlands to support macroinvertebrate taxa common to natural wetlands (although functionally they may be similar).

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