WETLAND RESTORATION IN THE NORTH DAKOTA PRAIRIE POTHOLE REGION: A MACROINVERTEBRATE COMMUNITY PERSPECTIVE

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Whitney Mariah Sauskojus

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Jon Sweetman
Co-Chair

Marinus Otte
Co-Chair

Christina Hargiss

Approved:

April 11th, 2024
Date

Craig Stockwell
Program Chair

ABSTRACT

This study assessed the long-term recovery of aquatic macroinvertebrate communities to wetland restoration. Previous research has suggested that even after a decade post restoration, macroinvertebrate communities may not fully resemble those of undisturbed reference sites, and how effective wetland restorations are in recovering macroinvertebrates is unclear. To assess macroinvertebrate recovery to restoration over long-time frames, thirteen restored and five reference wetlands were sampled in the North Dakota Prairie Pothole Region during July and August of 2019. Restored wetlands ranged from 20 to 32 years post-restoration, within restoration dates spanning between 1987-1999. Differences were examined between reference and restored sites, along with differences between four age categories: 20-26 years (n = 4), 29 years (n = 4), 31-32 years (n = 5) and reference sites (n = 5). No significant differences were found in aquatic macroinvertebrate richness and diversity between reference and restored wetlands, or among restoration age groups. Community composition was also similar among all restoration age groups, with no apparent influence from measured chemical and physical water variables and soil organic matter. These results suggest, within the Prairie Pothole Region, that restored wetlands contain diverse macroinvertebrate communities that resemble undisturbed reference sites after 20 to 32 years post-restoration.

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Most importantly, I want to thank my family. To great extent, my mom, grandma, and two sisters, who have always been my biggest cheerleaders. They continue to be my greatest inspirations through their strength, perseverance, and powerhouse personalities.

DEDICATION

To the women who endlessly love me for who I am, nurture my curiosity, build me up, and create an environment where anything is possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ETOH..Ethanol

HDPE..High density polyethylene

ND..North Dakota

PPR ....Prairie Pothole Region

WPA...Waterfowl Production Area

ANOVA ....Analysis of variance

NMDS..Non-metric multidimensional scaling

PERMANOVA ....Permutational analysis of variance

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

1. INTRODUCTION AND LITERATURE REVIEW

Wetlands are among the most valuable ecosystems in the world (Gascoigne et al 2011; de Groot et al 2012; Costanza et al 2014), providing many critical ecosystem services and functions such as carbon sequestration, flood protection and detention of runoff, waterfowl production, sedimentation reduction, and wildlife habitat (Johnson et al 2008; Doherty et al 2018). In addition, they improve water quality via sequestering nutrients such as nitrogen and phosphorus in sediments, serve as key sites for groundwater recharge and discharge (Johnson et al., 2008; Meyer & Whiles, 2008; Bortolotti et al., 2016), and provide an important area for human recreation (Montgomery et al 2021). Despite their value, significant wetland losses have occurred since the 1700's, with a net loss of 21% globally (Fluet-Chouinard et al 2023).

Ecological restoration provides an opportunity for various habitat types, biological communities, and ecosystem services to return to a state representing natural, pre-impacted conditions (Gann et al 2019). In recent decades, there has been a particular emphasis on wetland restoration in the Prairie Pothole Region (PPR) due in large part to the area's importance to migratory birds and waterfowl (Niemuth et al 2014). Within a 20 year period, countries in North America have spent over $70 billion to restore wetlands, yet rigorous evaluation of restoration success or failure have been limited, and monitoring post-restoration has been assessed only to a small degree (Moreno-Mateos et al 2012).

Understanding the response of key bioindicators can be beneficial to monitoring the effectiveness of wetland restoration. Macroinvertebrates are important in the function of freshwater ecosystems (Batzer and Boix 2016), and have proven to be useful indicators of water quality in lotic bioassessment programs for many decades (Cummins and Klug 1979; Xu et al 2014). However, studies have been less conclusive on their effectiveness as indicators of post-restoration condition in wetlands and few studies have examined long-term recovery of macroinvertebrate following restoration.

1.1. Prairie Pothole Region

The PPR is characterized by its collection of depressional wetlands, also known as 'potholes'. These potholes were formed in the late Pleistocene, beginning ~10,000 years ago, and were the result of glacial retreats of the Laurentide ice sheet (Johnson et al 2008). The PPR spans across three Canadian provinces (Alberta, Saskatchewan, and Manitoba) and five U.S. states (North Dakota, Iowa, South Dakota, Montana and Minnesota), with an area of ~770,000 km^2 (Doherty et al 2018)(Figure 1). Wetlands in the PPR play a crucial role in maintaining regional biodiversity by supporting a variety of wildlife and plant species, regulating ecosystem processes, and providing essential habitats (Doherty et al 2018). For example, wetlands within this region are vital for grassland birds, nesting waterfowl, and shorebirds, with ~50% of the North American migratory waterfowl population inhabiting them and one third utilizing them as breeding habitat (US EPA 2015).

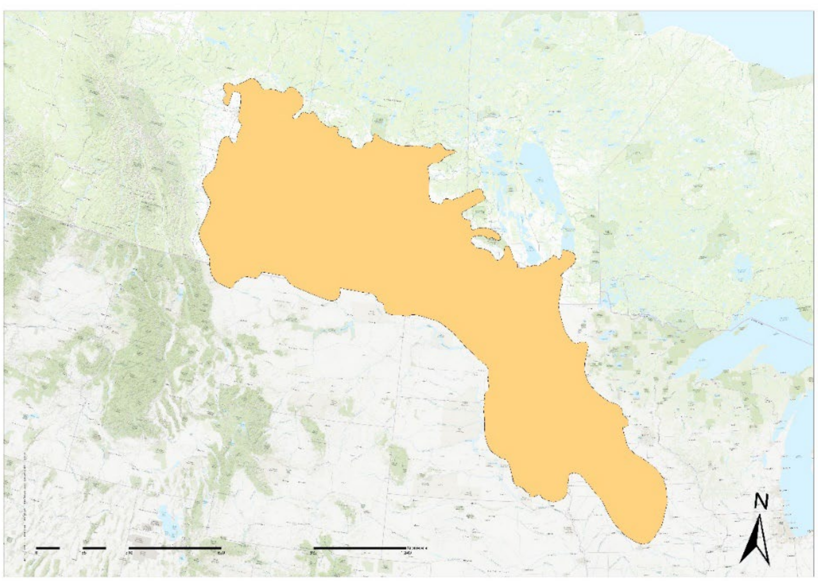

Figure 1. Map of the Prairie Pothole Region, within Canada and the United States.

The climate within the PPR is highly variable, generally semi-arid and characterized by its highly dynamic, interannual and regional cycles, with periods of drought and deluge (Karl and Riebsame 1984; Niemuth et al 2010; Mushet et al 2022). Wetlands in this region are sensitive to climate variability, which generates a variety of water levels, resulting in a wide range of wetland distributions and sizes. The majority of these prairie wetlands receive water from spring snowmelt, rainfall, or runoff from surrounding watersheds (LaBaugh et al 1998), and are typically an array of freshwater marshes (Dahl 2014).

1.2. Wetland Restoration

1.2.1. Wetland loss

Globally, the most extensive and rapid decline of wetlands occurred in the mid-20th century across China, the United States, and Europe (Fluet-Chouinard et al 2023), primarily due to agricultural expansion and development. Drainage of wetlands for agricultural conversion, in particular croplands, has resulted in a total loss of 61%, making it the main driver of natural wetland loss (Fluet-Chouinard et al 2023). Approximately 71% of the land use in the U.S. PPR is cropland (Rashford et al 2011). Unsurprisingly, the main mechanism of wetland degradation in North Dakota (ND) is agricultural development, amounting to a total loss of 2.7 million acres by the 1980's due to the drainage and filling of wetlands (Dakota Water Science Center 2017).

1.2.2. Federal, state, and local policy and regulation

Although there is no legislation that singularly addresses wetland loss, numerous policies have been implemented that affect wetlands. One of the most influential pieces of legislation occurred in 1972, the Clean Water Act, which was implemented as a comprehensive framework that includes provisions that target wetland restoration and protection (US EPA 2013a). Subsequently, additional efforts were enacted to help prevent wetland loss. One of which included the Wetland Conservation Provision of the Farm-Bill or “swamp buster”, created to incentivize farmers from converting wetlands to agriculture (Dahl 2014). Numerous other conservation programs and policies have been created to support wetland conservation and restoration efforts, including the Conservation Reserve Program, Wetland Reserve Program, North American Wetland Act, Conservation Reserve Enhancement Program, Environmental Quality Incentives Program, and Wildlife Habitat Incentives Program (Gleason et al 2011; Gascoigne et al 2011; Dahl 2014).

1.2.3. Restoration in the Prairie Pothole Region

With awareness of wetland loss and importance growing, state, federal, public, and private groups have increased restoration efforts, with thousands of attempts occurring every year in the US (Reid et al 2005). One of the main goals of wetland restoration is to re-establish wetland function and productivity to a state similar to natural, reference wetlands (Rader et al 2001). In the PPR, restoration efforts have been particularly prevalent, with the improvement of non-game bird and waterfowl habitat and water quality as the shared focus (Mulhouse and Galatowitsch 2003).

A variety of wetland restoration techniques have been used in the U.S (Knutsen and Jr 2001). Hydrologic alteration removes water from wetlands via tile breaks, ditching, and filling. The two most common techniques to restore these wetlands consists of a tile break, which removes a portion of an agricultural tile used to drain a wetland, and a ditch/drain plug, which impounds water using a dike or a berm (Knutsen and Jr 2001; Gleason et al 2011). Additionally, excavation and sediment removal, the most expensive and biologically invasive technique, is used to restore filled wetlands (Knutsen and Jr 2001)

1.2.4. Post-restoration monitoring and assessment

Wetland restoration is increasing, yet its success and efficacy post-restoration is still widely under-monitored and under-assessed (Moreno-Mateos et al 2012). Plant assemblages are among the most commonly used indices (Knutsen and Jr 2001; Guntenspergen et al 2002; Moreno-Mateos et al 2012). Vegetation recovery is slow and can take as long as 30 years to become close to reference level conditions, with complete recovery not being reached even 100 years after initial restoration (Moreno-Mateos et al 2012). In addition to wetland flora, bird communities have received generous attention within the PPR regarding post-restoration monitoring and research (Knutsen and Jr 2001). Physical and chemical characteristics of wetlands as well as other smaller biological indices, such as invertebrates, were far less studied (Knutsen and Jr 2001).

1.3. Aquatic Macroinvertebrates

Macroinvertebrates are important in the function of wetland ecosystems and contribute a vast majority of the biodiversity (Batzer and Wissinger 1996). They are food sources for birds (waterfowl, shorebirds, other water birds), fish, and other invertebrates, they contribute indirectly to nutrient transfer to higher level organisms by recycling detritus, and regulate key ecological functions such as nutrient cycling and primary production (Batzer and Boix 2016).

1.3.1. Dispersal abilities

Macroinvertebrates, especially those with high dispersal abilities, are known to rapidly colonize and recover from restoration relatively quickly (Delphey 1991; VanRees-Siewert 1993; Brown et al 1997; Meutter et al 2002; Bortolotti et al 2016). Higher connectivity between freshwater systems increases the chance of colonization (Meutter et al 2002). Poor dispersal ability is the main limitation for invertebrates not occurring or establishing as quickly in restored wetlands (Batzer and Wissinger 1996). Wetlands in the PPR tend to favor invertebrates with strong dispersal abilities due to the frequent drying and flooding periods (Bortolotti et al 2016).

1.3.2. Utilization as bioindicators

Macroinvertebrates are considered suitable study organisms, as they require easy, low-cost sampling methods, are taxonomically rich and well-described, have relatively sedentary and short life-spans, and are abundant and widespread (Balcombe et al 2005). In contrast, identification to species requires a higher skill level, which may take additional time and resources that may not be feasible for many projects. Some taxa, such as those in the order Ephemeroptera, Plecoptera, and Trichoptera, are particularly sensitive to ecological conditions such as water quality, and are frequently used in stream monitoring programs (US EPA 2013b).

Aquatic macroinvertebrates are the most commonly used assessment tool for monitoring function and productivity in streams, rivers, and lakes compared to any other group of organisms (McDonald et al 1991; Carter et al 2017), with community recovery in restored lotic systems being well understood (Covich et al 1999; Xu et al 2014). Utilizing them in wetland assessments, however, is less widespread, with little consensus between studies regarding their reliability as an indicator (Meyer and Whiles 2008; Marchetti et al 2010; Batzer and Ruhí 2013; Lu et al 2021). The amount of time it takes for macroinvertebrate communities to colonize restored wetland ecosystems in which they resemble communities of natural, reference wetlands is currently unknown (Moreno-Mateos et al 2012). Lu et al (2021) concluded that invertebrates begin to exhibit substantial recovery after 4 years of restoration, with restored wetlands having 50% of the taxa seen in natural wetlands, which could signify that they can be used as early indicators of restoration success and exhibit potential to be promising indicators. One meta-analysis (Moreno-Mateos et al 2012) collected invertebrate data from restored and created wetlands ranging up to 25 years post restoration. Communities began to statistically converge between 5 to 10 years, although they never reached reference level conditions even after 25 years post-restoration. With a limited amount of data and studies that have accessed longer post-restoration time-series compared to indicators such as plants (i.e., 100 years post-restoration), longer term studies could identify if communities reach reference level conditions.

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2. MACROINVERTEBRATE COMMUNITIES IN NATURAL AND RESTORED WETLANDS IN THE NORTH DAKOTA PRAIRIE POTHOLE REGION

2.1. Introduction

Over the last few decades, wetlands have become increasingly recognized as one of the most productive and valuable ecosystems pertaining to the services they provide (Costanza et al 2014). At the same time, the rate at which they have been lost has historically been high. The Prairie Pothole Region (PPR) has undergone considerable wetland destruction and degradation, with ~42% of the total area in North Dakota (ND) lost (Dakota Water Science Center 2017). Throughout the late 1980s, a surge of wetland restorations occurred across the PPR, and thousands of wetlands were left unmanaged to recover and virtually no monitoring occurred post-restoration, which eventually showed its limitations as invasive plant species began to overrun them (Galatowitsch and Van der Valk 1996). Currently, efficacy of restorations in North America have been measured by being compared to the structure and function of pre-impact, reference wetland conditions (Rader et al 2001), using various indicators (e.g., plant communities, waterfowl diversity, biogeochemistry) relevant and feasible to the project's scope and budget. Currently, however, assessment of a wetland restoration's success and condition over time is still limited, inefficient, or nonexistent (Moreno-Mateos et al 2012). Few studies focusing on macroinvertebrate communities have assessed older restored sites, specifically beyond 15 years post restoration, with most studies directing their attention to sites restored for 0-5 years prior (Moreno-Mateos et al 2012), limiting the amount of long-term data and therefore, advancements for future conservation and management planning. Macroinvertebrates have been frequently studied as part of assessing restored wetland condition (VanRees-Siewert 1993; Brown et al 1997; Stanczak and Keiper 2004; Balcombe et al 2005; Marchetti et al 2010;

Bortolotti et al 2016; Lu et al 2021), but a lack of conclusive results and long-term recovery data in wetland restorations (Batzer 2013; Gleason and Rooney 2017; McLean et al 2021) inhibits efficient assessment and planning of future restorations.

Over the last 40 years, wetland invertebrates have acquired considerable attention in North America (Batzer and Wissinger 1996; Bortolotti et al 2016). Wetland bioassessment programs began utilizing macroinvertebrates due to their high diversity, wide distribution and relatively sedentary lifestyles, cost-effective ease of sampling, and their ability to reflect environmental conditions (US EPA 2013). While their reliability and usefulness as indicators is less conclusive, previous studies have found that within 3 to 10 years, macroinvertebrate assemblages in restored sites are close to converging with those in reference wetlands (Moreno-Mateos et al 2012), representing highly diverse communities soon after restoration, owing to effective dispersal abilities (Delphey 1991; VanRees-Siewert 1993; Brown et al 1997; Meutter et al 2002; Bortolotti et al 2016). General macroinvertebrate community metrics, such as abundance, diversity, and richness are routinely analogous between reference and restored sites (VanRees-Siewert 1993; Brown et al 1997; Stanczak and Keiper 2004; Balcombe et al 2005; Marchetti et al 2010; Bortolotti et al 2016), while macroinvertebrate community composition reveals more complicated results that vary based on many biotic and abiotic factors (e.g., hydrological connectivity, climate, water chemistry, predation)(Meyer and Whiles 2008; Marchetti et al 2010; Batzer 2013; Kolozsvary and Holgerson 2016; McLean et al 2021).

2.1.1. Objectives

Due to the high value placed on wetlands in the Prairie Pothole Region (PPR), particularly for waterfowl production, there have been many wetland restoration projects conducted since the 1980s (Knutsen and Jr 2001), making the PPR an ideal area to assess the response of macroinvertebrate communities to past wetland restoration. The objectives were to 1) investigate differences in macroinvertebrate communities between restored and reference wetlands in the North Dakota portion of the PPR, and 2) investigate differences in macroinvertebrate communities in restored sites based on years since wetland restoration. It was hypothesized that macroinvertebrate communities within restored PPR wetlands would become more similar to communities in natural, reference wetlands as time since restoration increases. To assess this, macroinvertebrates were sampled in thirteen restored and five reference wetlands in the North Dakota PPR, with restoration having occurred between 1987 and 1999. General macroinvertebrate community metrics (i.e. diversity, richness, composition) were compared and changes in communities assessed as a function of time since restoration. This research contributes to the understanding of how aquatic macroinvertebrates respond to restoration, which could help improve the effectiveness and efficiency of monitoring and inform future management of wetlands post-restoration.

2.2. Materials and Methods

2.2.1. Site selection and study area

In total, eighteen wetland sites extending across eight counties (Towner, Cavalier, Ramsey, LaMoure, Goodrich, Stutsman, Wells, Hampden) in the North Dakota PPR were selected for sampling (Figure 2). Two types of wetlands were selected: natural, further referred to as reference wetlands (n = 5) and restored (n = 13). Both reference and restored wetlands were all located on protected areas including Wildlife Management Areas, Wetland Management Districts and Waterfowl Production Areas with surrounding land cover consisting predominately of native prairie habitat, so direct influence from agricultural activities was minimized. All restored wetlands were semi-permanent as they are inundated for consecutive years. Reference wetlands consisted of four semi-permanent and one temporary wetland (Table 1). Restored wetlands were previously drained by a ditch and underwent hydrologic restoration by the ditch plug method. To allow comparison between restored wetlands over time, we only examined this method and excluded those where sediment was excavated due to its potential to disturb benthic organisms (Smith et al 2016; Hassett and Steinman 2022) The years that restorations occurred at our study sites were 1987 (n = 2), 1988 (n = 3), 1990 (n = 4), 1993 (n = 1), 1994 (n = 1), and 1999 (n = 2), with time since restoration therefore ranging from 20 to 32 years.

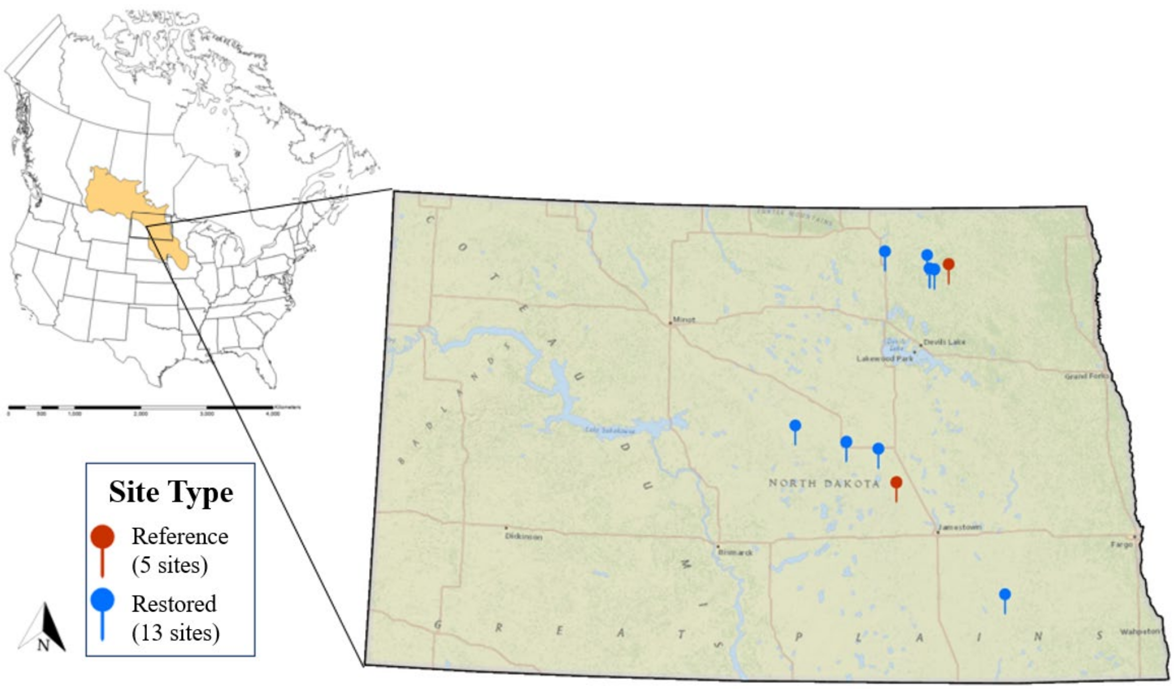

Figure 2. Map of the study region including locations of wetland sites (pins) sampled within the PPR (yellow shading) in North Dakota during the summer of 2019. There are two site types, restored (blue pin) and reference (red pin). Due to the close proximity of many of the sites among wetland complexes, some pins may overlap. Refer to Table 1 for further site descriptions and wetland coordinates.

Table 1. Site information for 13 restored and 5 reference wetlands sampled for macroinvertebrates in 2019 within the ND PPR.

Wetland Type	Wetland Name	Year Restored	Years Post Restoration	County	Latitude	Longitude	Permanence Class
Restored	Nikolaisen 1	1988	31	Towner	48.584444	-99.215556	Semi-permanent
	Nikolaisen 5	1988	31	Towner	48.585000	-99.218333	Semi-permanent
	Banner South 19	1994	25	Cavalier	48.560556	-98.805278	Semi-permanent
	Kneeling Moose 1	1990	29	Ramsey	48.475278	-98.783056	Semi-permanent
	Kneeling Moose 2	1990	29	Ramsey	48.475000	-98.778889	Semi-permanent
	Mellin 2	1999	20	Ramsey	48.470000	-98.734722	Semi-permanent
	Mellin 10	1999	20	Ramsey	48.466944	-98.735556	Semi-permanent
	Pilgrim's Rest 5	1993	26	LaMoure	46.377222	-98.083889	Semi-permanent
	Dense Nesting Cover 7	1988	31	Goodrich	47.460833	-100.068806	Semi-permanent
	Hawk's Nest 1	1987	32	Stutsman	47.315278	-99.273889	Semi-permanent
	Hawk's Nest 2	1987	32	Stutsman	47.316389	-99.274722	Semi-permanent
	Sweetgrass 1	1990	29	Wells	47.360000	-99.579167	Semi-permanent
	Sweetgrass 2	1990	29	Wells	47.356389	-99.581389	Semi-permanent
Reference	Cottonwood SSA T1	--	--	Stutsman	47.098611	-99.102500	Temporary
	Cottonwood SSA P8	--	--	Stutsman	47.099167	-99.103333	Semi-permanent
	Cottonwood SSA P3	--	--	Stutsman	47.101944	-99.101667	Semi-permanent
	Phil Aus WPA 2	--	--	Hampden	48.502392	-98.594803	Semi-permanent
	Phil Aus WPA 1	--	--	Hampden	48.500728	-98.596133	Semi-permanent

2.2.2. Data collection and laboratory processing

Benthic macroinvertebrates were sampled during the months of July and August in 2019. Each site was sampled once each month, with a total of 36 samples collected in the duration of the project. To ensure a representative sample of the population was obtained, three subsamples per site were collected during each sampling period and composited from each wetland in an area of emergent vegetation and when present, floating-leaf vegetation, submerged vegetation, and open water (Figure 3; MPCA 2015). Samples were collected using a 500µm mesh D-frame net held slightly above the substrate, whereafter a sweep netting motion was performed continuously in a 2-minute time span. During this time, the collector would walk forward in a zigzag pattern, occasionally tapping on the substrate with the net or foot to stir up benthic macroinvertebrates, while also maintaining an up and down motion with the net to ensure sampling of the entire water column. This method has been shown to be an effective means to sample aquatic macroinvertebrate richness in wetlands (MPCA 2014) but may underrepresent active swimming taxa or night active predators. If there was extensive debris collected in the process, debris was flushed with water, checked thoroughly for macroinvertebrates and removed from the sample. The contents of the sample were then transferred into a bucket, poured into a 500µm sieve, washed thoroughly using a squeeze bottle filled with water from the sampled wetland, and transferred into labeled 1L high density polyethylene (HDPE) sample bottles filled with 95% ethanol (ETOH) for proper preservation.

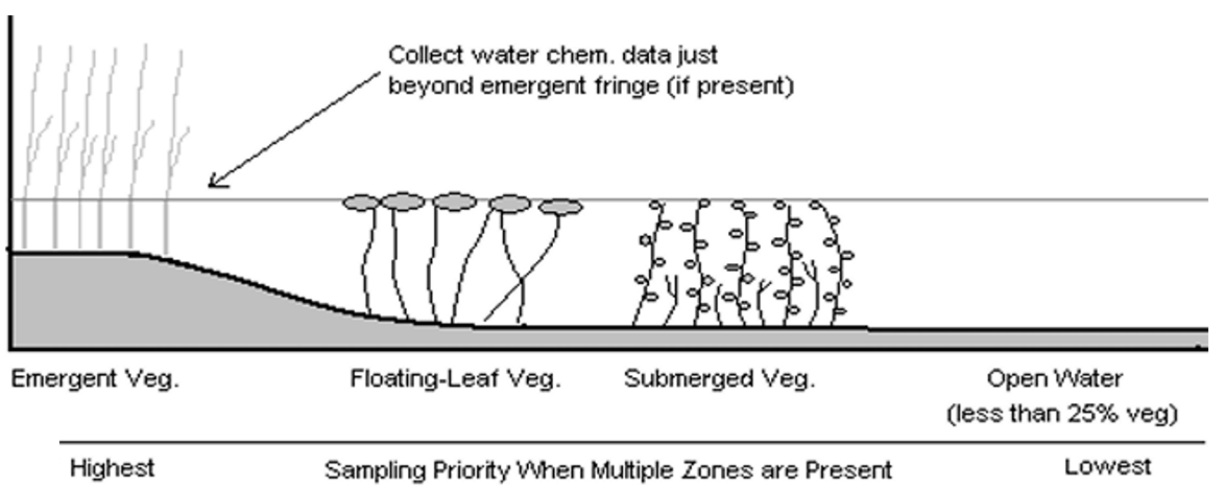

Figure 3. Diagram representing the 4 sampling zones potentially present within wetlands, with priority of sampling for macroinvertebrates ranging from highest to lowest; collection of water chemistry samples collected along the emergent fringe (MPCA 2015).

Invertebrate samples were transported to the lab, where they were picked from detritus using forceps, identified, and subsequently sorted into 80 mL plastic containers with 70% ETOH preservative. Using multiple dichotomous keys (Merrit & Cummins 1996; McCafferty 1999; VCSU), macroinvertebrates were identified down to genus, when possible, to quantify taxon abundance. To ensure quality control, voucher specimens were sent to an independent taxonomist to be reanalyzed (EcoAnalysts Inc, Idaho, USA).

At each site prior to macroinvertebrate sampling, surface water was collected at three separate locations within a wetland just beyond the emergent fringe to analyze water nutrients and ions (MPCA 2015). A multimeter probe (YSI Inc, USA) was used ~10cm below the water's surface to measure water temperature, conductivity (EC), and pH. In addition, a labeled 1L HDPE Amber Nalgene sample bottle was submerged upside down just below the surface (5-10cm). Water samples were stored immediately on ice, transported to the lab and stored at 4°C. Within 2 days, using a 900mL Erlenmeyer flask fitted with a vacuum pump, samples were filtered through syringe-mounted 0.45 µm filters (25mm diameter membrane filter). After each bottle was rinsed with water from the associated wetland, three 125mL polyethylene bottles (2 filtered for total dissolved phosphorus (TDP) and dissolved organic carbon (DOC), and 1 unfiltered for total organic carbon (TOC)) and one 500mL HDPE Amber Nalgene bottle (unfiltered for total phosphorus (TP), total nitrogen (TN), calcium (Ca^{2+}) were submitted to the NDSU Soil Laboratory (Fargo, ND) for analysis of total Kjeidahl nitrogen TKN, TP, TDP, TOC, DOC, Ca^{2+}, magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), and sulfate (SO_4^{2-}). Unfortunately, water samples were not collected at three of the sites (Banner South 19, Phil Aus WPA 1 and 2) and were excluded from related analyses. Five soil samples were collected in the field along the perimeter of the wetland, and loss-on-ignition as an estimate of organic matter (OM(%)) content in soils was determined in the lab (Combs and Nathan 2012).

2.2.3. Data analyses

2.2.3.1. Taxonomic richness and diversity

Statistical analyses were performed using R (R Core Team 2022; version 4.22). Macroinvertebrate abundances were averaged between the samples taken in July and August of 2019, and were used to calculate taxa richness and diversity (Shannon-Wiener index (H; Shannon & Weaver 1948). Richness and diversity measures were calculated using the ‘specnumber’ and ‘diversity’ functions in the *vegan* package 2.6-4, respectively (Oksanen et al 2022). Assumptions of normal distribution were tested using the ‘shapiro.test’ function in the *rstatix* package 0.7.2 (Kassambra 2023), and homogeneous variances were tested using the ‘leveneTest’ function in the *car* package 3.0-5 (Fox & Weisberg 2019). An analysis of variance (ANOVA) was used to identify significant differences in average taxa richness and diversity among the two wetland types, reference and restored. This analysis was conducted using the ‘aov’ function in the *stats* package (R Core Team 2022). To visualize differences, boxplots were created using the ‘ggboxplot’ function in the *ggpubr* package 0.6.0 (Kassambra 2023).

To explore differences in richness and diversity across restoration age, restored wetlands were categorized into four age categories or bins. These were based on years passed since restoration relative to the time of sampling: reference (n = 5), 20-26 years (n = 4), 29 years (n = 5), and 31-32 years (n = 5). Groups were chosen based on having a more evenly distributed sample size within each group. An ANOVA was used to identify significant differences in average taxa richness and diversity among those groups.

Indicator species analysis was used to determine the relationship between species relative abundances and the four ages bins. Indicator values range from 0 to 1, with a value closer to 1 indicating a stronger association to the specific age group. This analysis was run using the using the 'multipatt' function in the R *indicspecies* package 2.6-4 (De Cáceres M 2009).

2.2.3.2. Community composition

To visualize differences in macroinvertebrate community composition across restored wetland ages, each site was grouped according to the four age bins, and a non-metric multidimensional scaling (NMDS) ordination was performed using the 'metaMDS' function in *vegan* package 2.6-4 (Oksanen 2022). As invertebrate data consisted of many rare taxa, data were square root-transformed prior to multi-variate analyses to reduce the impacts of dominant taxa. The distance measure used for the scaling was a Bray-Curtis dissimilarity matrix. Due to the lack of water data for three sites, two separate ordinations and analyses were performed: one with all eighteen sites without environmental variables and one with fifteen sites fitted with environmental variables. The scree plot of stress versus dimensionality determined that a three-dimensional NMDS (Appendix A; Figure A2) and a two-dimensional NMDS (Appendix A; Figure A2) should be used for data without and with environmental variables fitted, respectively. For both ordinations, a permutational analysis of variance (PERMANOVA) was conducted to statistically compare community differences among restoration age bins using the 'adonis2' function in the *vegan* package 2.6-4 (Oksanen et al 2022). Environmental variables were fit with the NMDS ordination using the 'envfit' function in *vegan* package 2.6-4 (Oksanen et al 2022) to determine if there were any driving factors influencing macroinvertebrate community differences.

2.3. Results

2.3.1. Taxonomic richness and diversity

Across all wetland sites, a total of 67 invertebrate taxa were identified belonging to 36 different families (Appendix A; Table A1). The most abundant taxa in reference wetlands were, *Chaoborus*, *Hyalella*, and *Anax*, while Chironomidae, Corixidae, *Armiger, Physa, Promenetus, Gyraulus, Nehalennia*, and *Callibaetis* were more abundant in restored wetlands (Appendix A; Figure A3 and A4). In restored wetlands, the average taxa richness ranged from 12 to 31 taxa and Shannon's Diversity (H) ranged from 0.64 to 2.44 (Figure 4). In reference wetlands, the average taxa richness ranged from 15 to 34 taxa and diversity Shannon-Weiner Index (H) ranged from 0.61 to 2.45 (Figure 4). Two common gastropod families (i.e., Physidae, Planorbidae) were evenly distributed among reference and restored sites (Appendix A; Figure A3).

No significant differences were observed between restored and reference wetlands in either diversity (Shannon-Weiner Index) (Figure 4A: ANOVA, $F = 0.002$, $p = 0.962$) or taxonomic richness (Figure 4B: ANOVA, $F = 0.037$, $p = 0.85$).

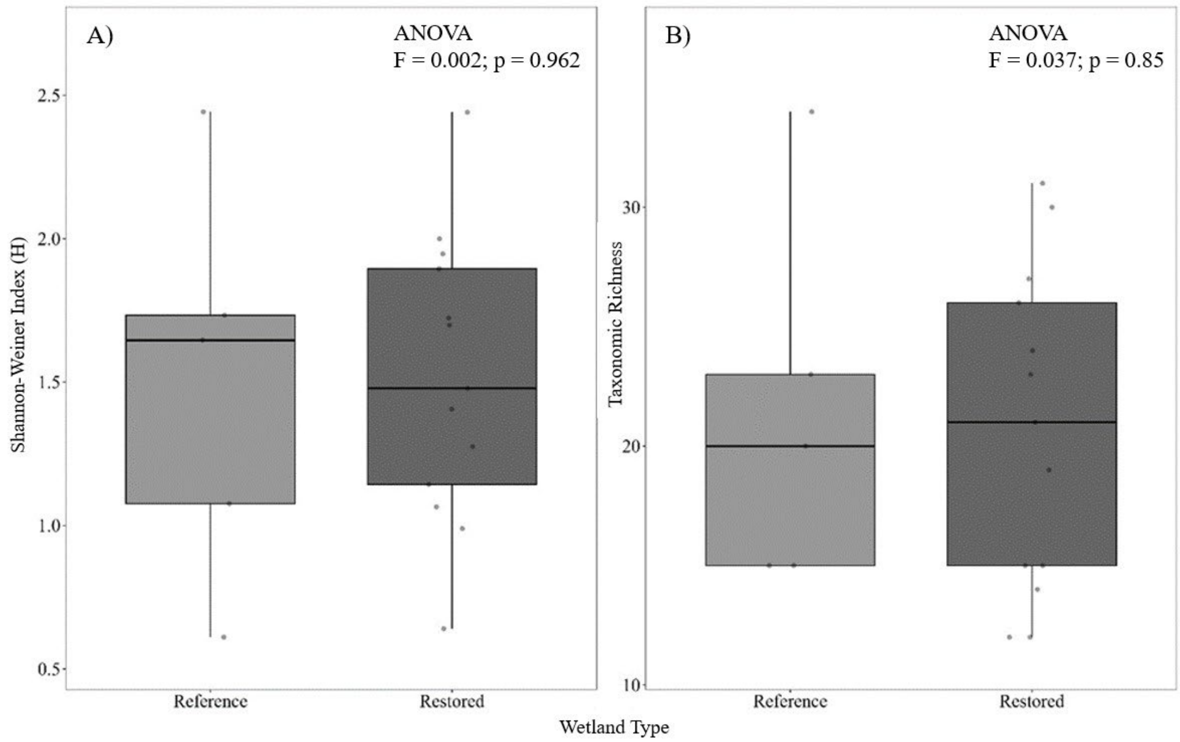

Figure 4. Macroinvertebrate diversity (Shannon-Weiner Index) (A) and taxonomic richness (B) in restored (n = 13) and reference (n = 5) wetlands. Points represent individual sites.

When restored wetlands were split into restoration age groups, there were also no significant differences observed between reference wetlands and across restoration age in either diversity (Shannon-Weiner Index) (Figure 5A: ANOVA, F = 0.478, p = 0.703) or taxonomic richness (Figure 5B: ANOVA, F = 0.678, p = 0.58).

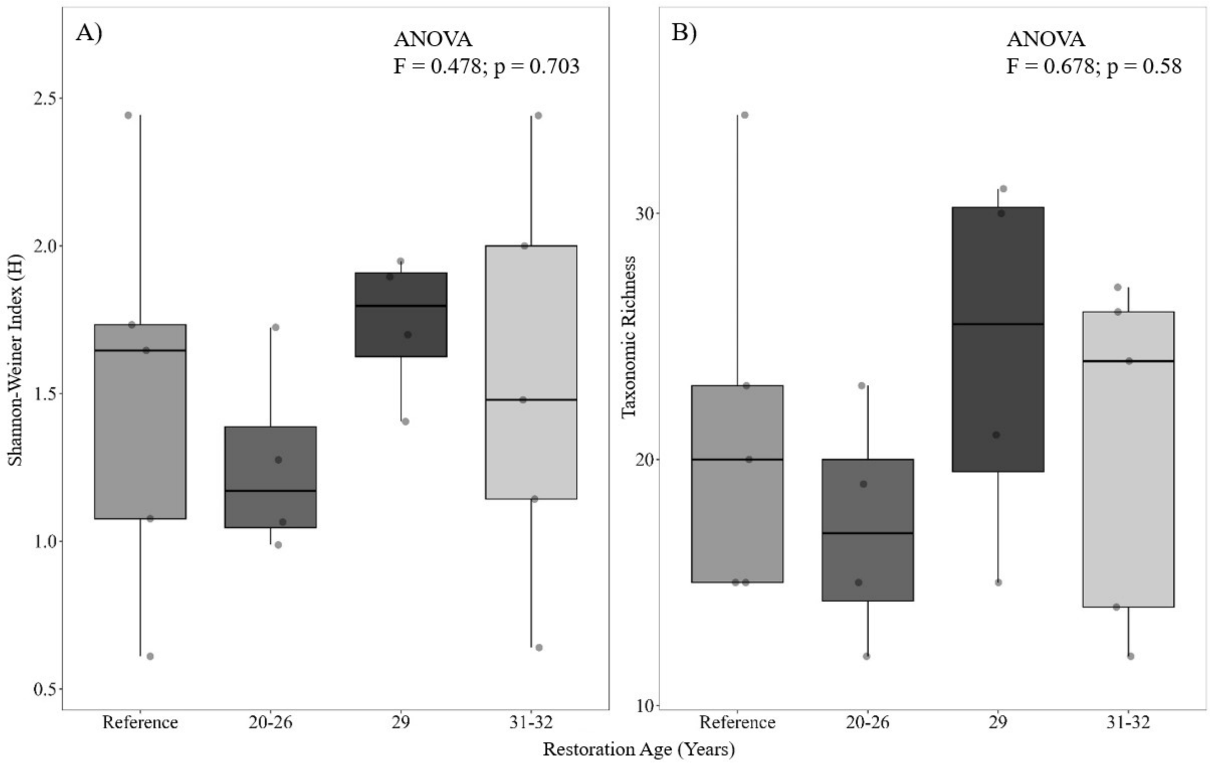

Figure 5. Macroinvertebrate diversity (Shannon Wiener Index) (A) and taxonomic richness (B) observed across restoration age. Groups were chosen based on obtaining the most evenly distributed amount of sampling sites possible: reference (n = 5), 20- 26 (n = 4), 29 (n = 4), and 31-32 (n = 5). Points represent individual sites.

Indicator species analysis (ISA) identified two taxa associated with the two oldest restoration age bins (Table 2); *Agabus* spp. in wetlands restored 29 years ago, and *Trichocorixa* spp. in wetlands restored 31-32 years ago. No other indicator species were identified differentiating reference and restored aquatic invertebrate communities.

Table 2. ISA species analysis for restored wetlands over a chronosequence.

Taxa	Restoration age (years)	Indicator value	p-value
Agabus spp.	29	0.791	0.015
Trichocorixa spp.	31-32	0.773	0.046

2.3.2. Community composition

The NMDS revealed a lack of clustering and extensive overlap among our reference sites and restoration age bins along the three ordination axes (Figure 6; 3D NMDS stress = 0.10). Additionally, PERMANOVA revealed no significant differences in macroinvertebrate community composition among restoration age bins (PERMANOVA: $F = 1.05$, $p = 0.37$; Figure 6). When water chemistry variables were fitted to a two-dimensional NMDS ordination (Figure 7; 2D NMDS stress = 0.16) there were also no significant differences between our age bins, and the fitted variables displayed a weak influence on community composition (PERMANOVA: $F = 0.95$, $p = 0.56$; Figure 7). None of the measured environmental variables significantly explained the variation in wetland macroinvertebrate community composition (Table 3).

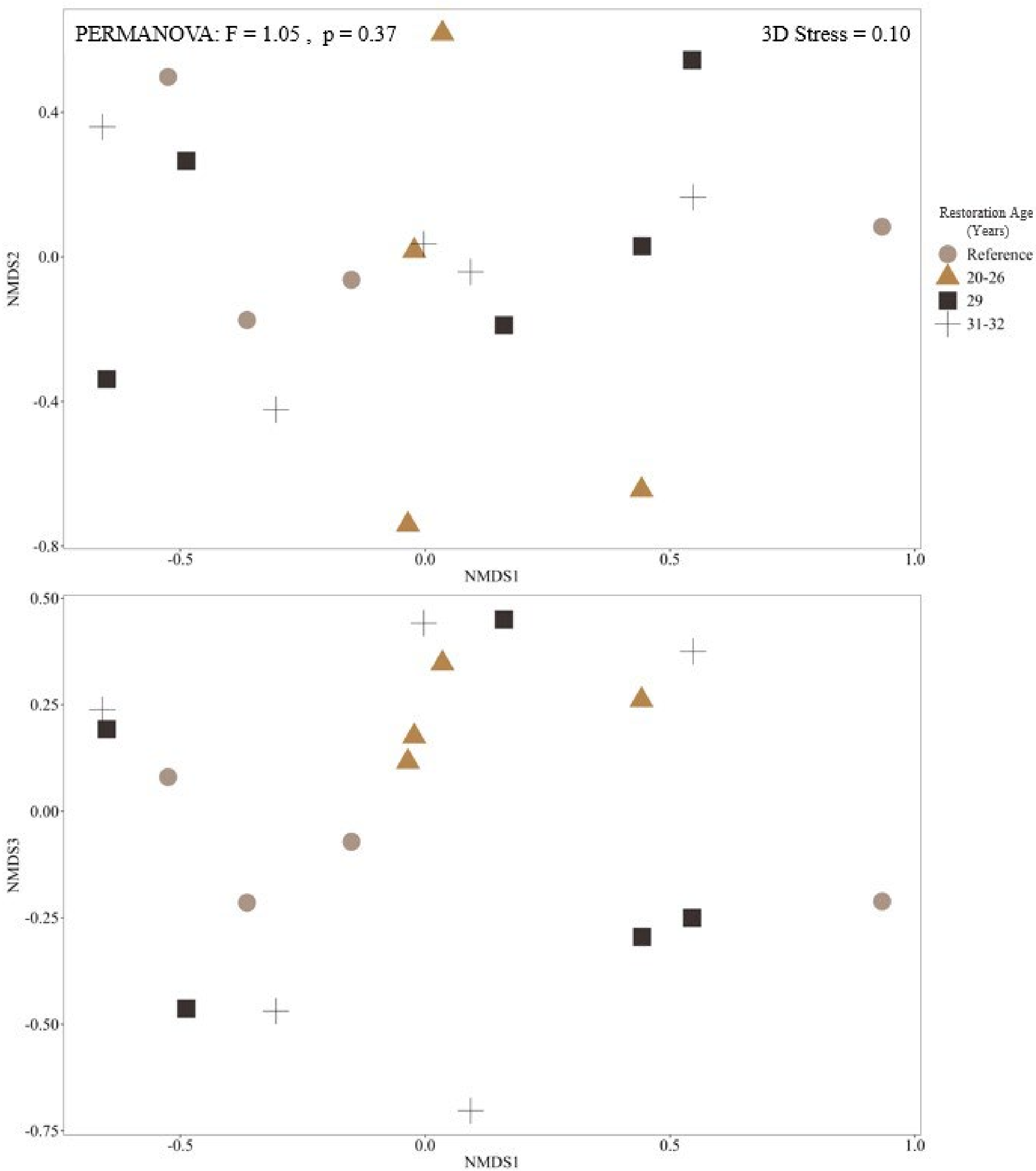

Figure 6. NMDS ordination of aquatic macroinvertebrate community composition in reference wetlands (n = 5) and restored wetlands categorized based on restoration age (20- 26 (n = 4), 29 (n = 4), and 31-32 (n = 5)). (PERMANOVA: F = 1.05, p = 0.37; 3D stress = 0.10).

Table 3. NMDS plot scores for all environmental variables measured for the 15 wetland sites.

NMDS1	NMDS2	Environmental Variables	*p*-value
-0.00997659	0.23050837	pH	0.756
-0.03702767	-0.19795809	EC (μS cm^{-1})	0.795
-0.01793554	-0.06832870	Temperature (°C)	0.958
-0.08742519	-0.00622577	OM (%)	0.941
-0.01727003	-0.45284434	TKN (mg L^{-1})	0.253
-0.12886074	0.11215197	TP (μg L^{-1})	0.842
-0.11724138	0.05636606	TDP (μg L^{-1})	0.897
0.16132323	0.39775116	Ca^{2+} (mg L^{-1})	0.305
-0.07544720	0.36336327	Mg^{2+} (mg L^{-1})	0.545
0.40055594	-0.07745477	Na^{+} (mg L^{-1})	0.328
0.30391517	0.43786200	K^{+} (mg L^{-1})	0.123
0.19253083	-0.31467099	Cl^{-} (mg L^{-1})	0.406
0.03014075	0.09101664	SO_4^{2-} (mg L^{-1})	0.953
0.23297426	0.09779306	TOC (mg L^{-1})	0.647

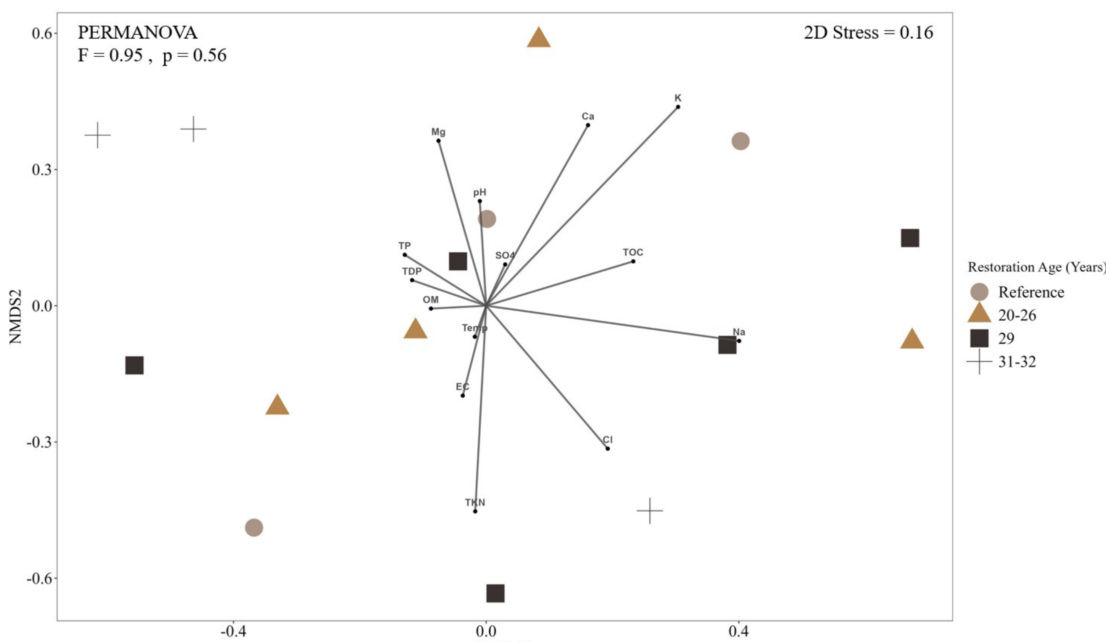

Figure 7. NMDS ordination of aquatic macroinvertebrate community composition in reference wetlands (n = 3) and restored wetlands categorized based on restoration age (20- 26 (n = 3), 29 (n = 4), and 31-32 (n = 5)), fitted with environmental variables (PERMANOVA: F = 0.95, p = 0.56; 2D stress = 0.16). Three wetlands (1 restored, 2 reference) were inadvertently not sampled for water quality in 2019, therefore not included in this ordination.

2.4. Discussion

This study examined the restoration success of restored wetlands in comparison to natural, reference wetlands in the Prairie Pothole Region by evaluating macroinvertebrate diversity, richness, and composition in sites restored at different time periods (i.e. 1987-1999). Contrary to our initial hypotheses, we did not find a significant difference in invertebrate taxa richness and diversity between restored and reference sites and among restoration ages. Similarly, neither how long the wetland had been restored, nor any of our measured environmental variables, were a significant predictor of invertebrate community composition. Several other studies investigated macroinvertebrate responses to wetland restoration in comparison to reference wetlands, reporting similarities in richness and diversity and concluding that diverse communities were present between 3 to 10 years post-restoration (VanRees-Siewert 1993; Brown et al 1997; Stanczak and Keiper 2004; Balcombe et al 2005; Stewart and Downing 2008; Meyer and Whiles 2008; Marchetti et al 2010; Sartori et al 2015; Bortolotti et al 2016; Coccia et al 2016). Within prairie wetland communities, Bortolotti et al (2016) found that macroinvertebrate communities in restored wetlands were not significantly different than reference wetlands 10 years post restoration. Our results, supported by the aforementioned studies, suggest that macroinvertebrate communities show evidence of recovery in what appears to be well before the 20–32-year period in our study.

While restored wetlands do seem to converge with natural wetlands in the PPR based on general community metrics (e.g., richness, diversity), the assessment of community assemblages and how they react to environmental conditions can prove to be complicated and unpredictable (Meyer and Whiles 2008; Marchetti et al 2010; Batzer 2013; Kolozsvary and Holgerson 2016; Lu et al 2021), even as restoration age increases. Fish predation/presence is noted to be one of the main drivers of invertebrate composition (Tangen et al 2003; Maurer et al 2014), attributed to declines in invertebrate abundance and diversity due to alterations in trophic states (i.e. turbidity). In our sites, no fish were observed during sampling, although, we did not extensively survey for fish presence. Water chemistry, the most assessed parameter in freshwater management, is known to influence macroinvertebrate community composition (Xu et al 2014). Despite this, none of the water chemistry variables measured in this study were significant predictors of composition. One explanation could be that water chemistry has also recovered, aligning with findings of Bortolotti (2016), where within 10 years, water chemistry of hydrologically restored PPR wetlands closely resembled that of natural wetland sites.

Restoration age may have contributed to the similarities observed in our results. Restored sites within this study range across a chronosequence of time, but only include wetlands restored 20 to 32 years ago, therefore, we might not have captured differences from more recent restorations (i.e., 0 to 19 years). Additionally, various wetland restoration techniques exist within the PPR (Knutsen and Jr 2001). Excavation of surface sediments removes egg and seed banks of biological species such as plants and benthic invertebrates (Smith et al 2016; Hassett and Steinman 2022). Ditch plug or fill techniques do not disturb benthic sediments, thus keeping both macroinvertebrate and vegetation seed banks intact. We specifically chose to focus on restorations that did not include sediment excavations. The removal of sediment would likely have a significant influence on benthic macroinvertebrates, and restorations using this approach may take much longer for aquatic invertebrate communities to recover. This could have potentially given the sites within our study an advantage over other techniques, being able to colonize much faster and with less spatial constraint. Schultz et al (2020), however, found that differences in restoration techniques did not alter macroinvertebrate communities. Comparisons of benthic macroinvertebrate communities from more recent restorations and those using different restoration approaches may be an important next step to evaluate biological recovery.

Macroinvertebrates are known to be rapid colonizers of restored sites (Delphey 1991), with many taxa having active dispersal abilities, such as flying adult stages, allowing them to recolonize quickly (Batzer and Wissinger 1996). The two indicator species that were associated with the two oldest restoration age bins, *Agabus* spp. (wetlands 29 years old post restoration) and *Trichorixa* spp. (wetlands 31-32 years old post restoration), were both taxa with aerial dispersal abilities/active flying adult stages. The lack of active dispersal abilities is often considered to be the main limitation affecting recovery (Batzer et al 1993) with flightless dispersers often taking longer to colonize (Batzer and Wissinger 1996). Curiously, various Gastropod taxa (i.e., *Armiger, Physa, Promenetus, Gyraulus*), known to be flightless invertebrates, occurred at the highest abundance within restored sites. Hydrological connectivity and permanence plays an important role in invertebrate dispersal (Batzer and Ruhí 2013). Colonization strategies for flightless invertebrates such as gastropods often include desiccation resistance (i.e., deposition of drought resistant eggs or spores within sediments). Restored wetlands within our study are all discharge, semi-permanent wetlands, which are fed through groundwater, meaning that though sites were drained, soil most likely remained consistently saturated even if surface water was not present. Additionally, Stanczak and Keiper (2004) found that adjacency to other wetlands could be a means of invertebrate colonization. Wetlands in this study were often adjacent (i.e., within the same wetland complex), therefore the close proximity to other wetlands most likely aided in quicker recovery, favoring invertebrates that can fly.

Overall, invertebrates in our study lacked any observable differences in richness and diversity and did not show any clear separation in composition among our sites. This could suggest that in older PPR restored sites (i.e., 20 to 32 years post-restoration), macroinvertebrate communities have recovered. Macroinvertebrate assemblages in wetlands have often been described as idiosyncratic (i.e., unpredictable), a conclusion supported by Batzer (2015), making them difficult to replicate and interpret. McLean et al (2021) examined a 24-year record of macroinvertebrate communities in relatively undisturbed North Dakota wetlands and found the drivers resulting in changes in macroinvertebrate communities were highly complex. The complex response of macroinvertebrates to environmental change may make it more of a nuisance than a useful tool for management and monitoring. However, it remains crucial to consider their role in maintaining and structuring freshwater ecosystems. Therefore, their use as standalone indicators may prove unnecessary or ineffective and may be contingent on the goal of the restoration project.

Additionally, although identifying to genus-level taxonomy yields new or supplementary information on assemblage-environment relationships (King and Richardson 2002), it could prove to be unnecessary within bioassessments in the PPR as family level results yielded similar results within our study. Family-level identification could be a more cost-effective option within bioassessments (Bailey et al 2001), that also reduces identification error that is more likely to occur past family level, and is generally the most useful when assessing ecological functions that are provided by certain taxa (Batzer and Ruhí 2013). However, we did not identify certain taxa to genus level, such as Chironomidae, a taxon highlighted for their importance in freshwater systems (Batzer et al 2006) and wetland bioassessments due to comprising a large proportion of total species (King and Richardson 2002; Jones 2008).

Climate is one of the key controls of how macroinvertebrate assemblages are structured (Batzer and Ruhí 2013; McLean et al 2021). Climate change and its associated increase in temperature will make wetlands in the region vulnerable to changes in hydrology and drying (Niemuth et al 2014), and is anticipated to alter aquatic macroinvertebrate communities largely due to their dependence on water permanency (Epele et al 2022). Over the last 20 years, the PPR has experienced a shift to a much wetter period with increasing precipitation (McKenna et al 2017). This shift could have inherently increased connectivity in our study via altered hydroperiods and contributed to a quicker and easier recovery of macroinvertebrates. Future climate change may result in a shift to drier warmer conditions, resulting in less wetlands on the landscape and alterations in wetland characteristics (i.e. hydroperiod, water chemistry) and perhaps increased barriers to recovery (McLean et al 2016; Epele et al 2022), especially in an already fragmented landscape.

Overall, our study suggests that hydrologically restored wetlands can replicate some aspects of reference wetland structure and function (i.e., aquatic macroinvertebrates), although additional ecological parameters (i.e. biogeochemistry, plant communities) may only reach 80% of reference conditions (Moreno-Mateos et al 2012). The results of this study provide additional information on how macroinvertebrates respond to wetland restoration in the ND PPR, taking into consideration that response to ecological conditions proves to be complex and inconclusive.

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3. CONCLUSIONS

3.1. Research findings

Contrary to our initial expectations, aquatic macroinvertebrate communities in our restored wetland sites were not significantly different than those of our reference sites. We also did not identify any significant drivers of macroinvertebrate community assemblages. Macroinvertebrate assemblages were variable across our sites, but differences in water chemistry or restoration status or age did not explain this variability. Often, due to the variability among wetland habitats, invertebrate communities respond in ways that are unpredictable (Batzer and Ruhí 2013). Our study was restricted to 13 restored sites, all which had been restored over 20 years ago, and five reference sites. Increasing our sample sizes to include additional wetlands (both undisturbed and restored) may help distinguish differences between undisturbed and reference sites.

3.2. Significance and conclusions

Restored Prairie Pothole Region (PPR) wetlands contain diverse aquatic macroinvertebrate communities after 20 to 32 years post-restoration. This research provides evidence that macroinvertebrate communities may be recovered in restored wetlands within the PPR, an important finding that contributes to the lack of literature assessing macroinvertebrate recovery in older restored wetlands (i.e. 15+). Aquatic macroinvertebrates are important in the functioning of wetland ecosystems due to their ability to cycle nutrients, their role in the food web, and their overall contribution to biodiversity (Batzer and Boix 2016). Within the PPR, particular emphasis is placed on macroinvertebrates role as a food source for waterfowl, with many restorations funded and supported due to the region's importance in the production (i.e. breeding habitat) and conservation of waterfowl (Knutsen and Jr 2001; Niemuth et al 2014). If one of the primary goals of restoration is to restore macroinvertebrate diversity and overall food web structure, analyzing macroinvertebrates has been recommended (Rader et al 2001). Utilizing macroinvertebrates as an indicator of restored wetland condition can provide valuable input in accordance with the respective restoration objectives, and incremental advancements, such as those resulting from this study, contribute to the breadth of information on management practices.

3.3. Future work

Community measures from a taxonomic perspective such as abundance, diversity and richness are typically similar between restored and natural wetlands, whereas functional traits are less conclusive and less studied (Meyer and Whiles 2008; Coccia et al 2021; Kohlmann et al 2021). Functional traits are biological traits that affect fitness and performance, which can have the ability to affect how an ecosystem is functioning. Macroinvertebrates have a wide range of functional traits, and expanding past taxonomic diversity can provide supplementary insights (Coccia et al 2021). One of the most well-studied traits, functional feeding groups (FFG), were initially created as a tool to use in studies of aquatic systems (Cummins 1973; Cummins and Klug 1979), with understanding the role of macroinvertebrates in ecosystem functions and how they consume resources as its main goal. FFG composition can be used as an indicator of change or disturbance in an ecosystem and its recovery (Ramírez and Gutiérrez-Fonseca 2014).

3.4. References

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APPENDIX. SUPPLEMENTARY TABLES AND FIGURES

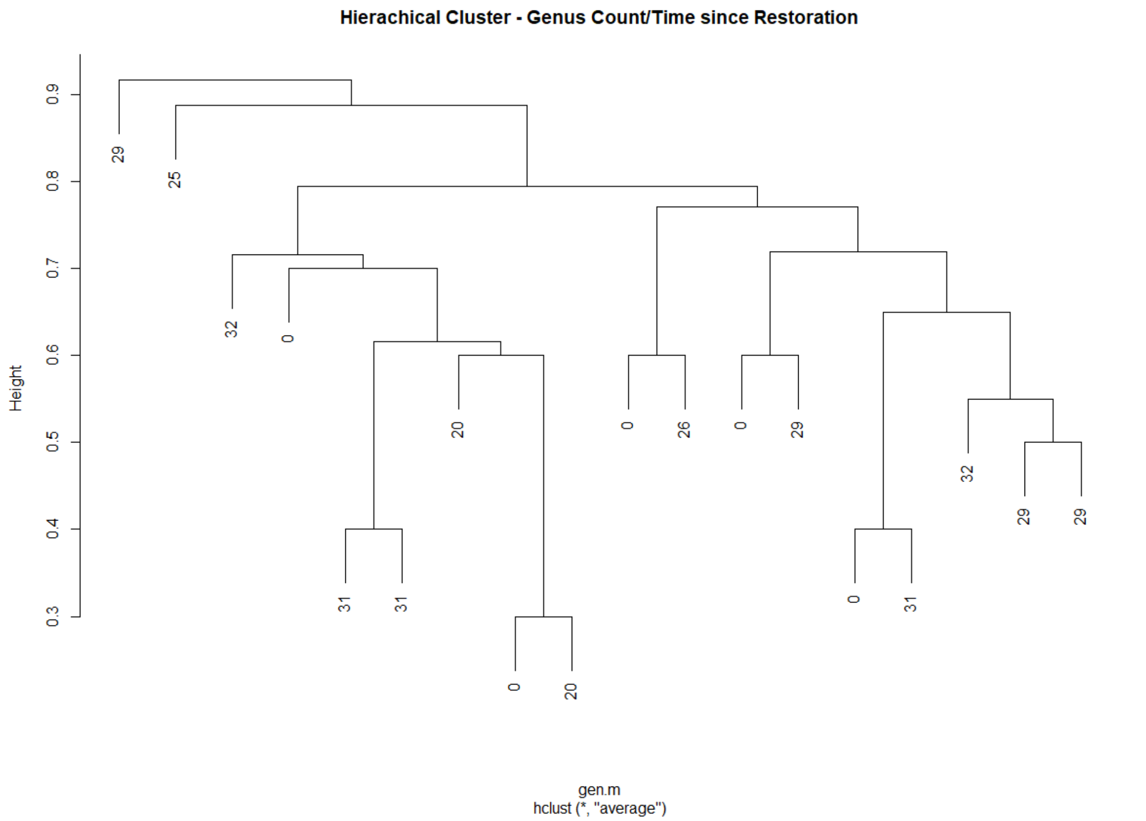

Figure A1. Hierarchical cluster analysis (Bray-Curtis method) for the 18 sites.

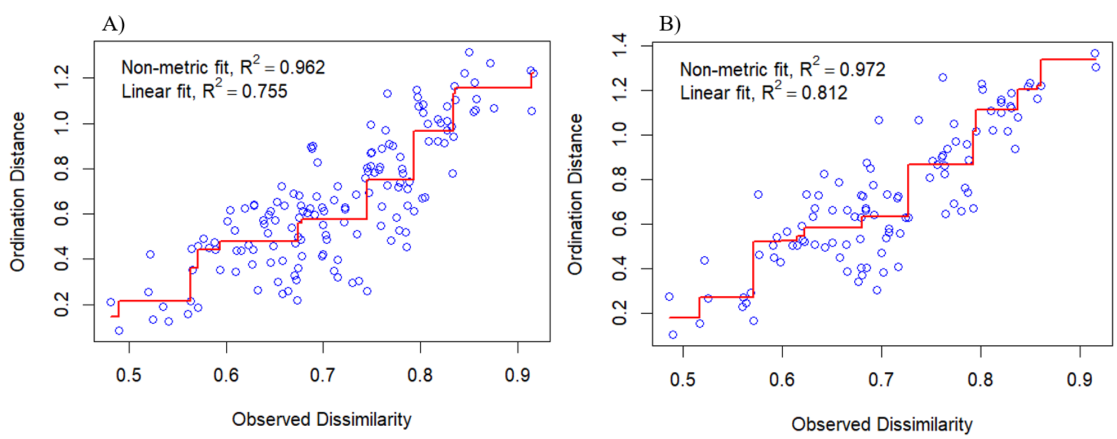

Figure A2. Stress plots of A) 18 sites and B) 15 sites with fitted water variables.

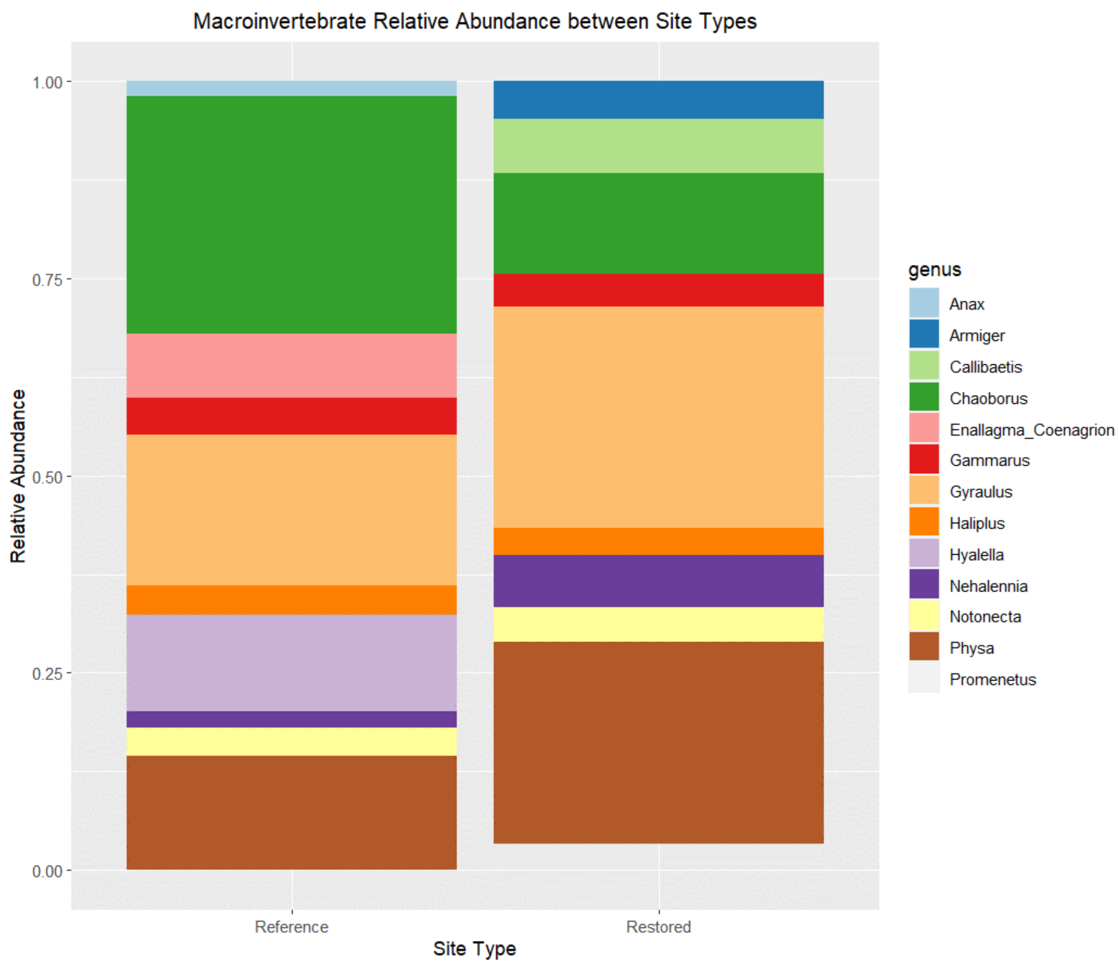

Figure A3. Macroinvertebrate relative abundance based on genus-level taxonomy between site types (reference and restored) for all 18 sites.

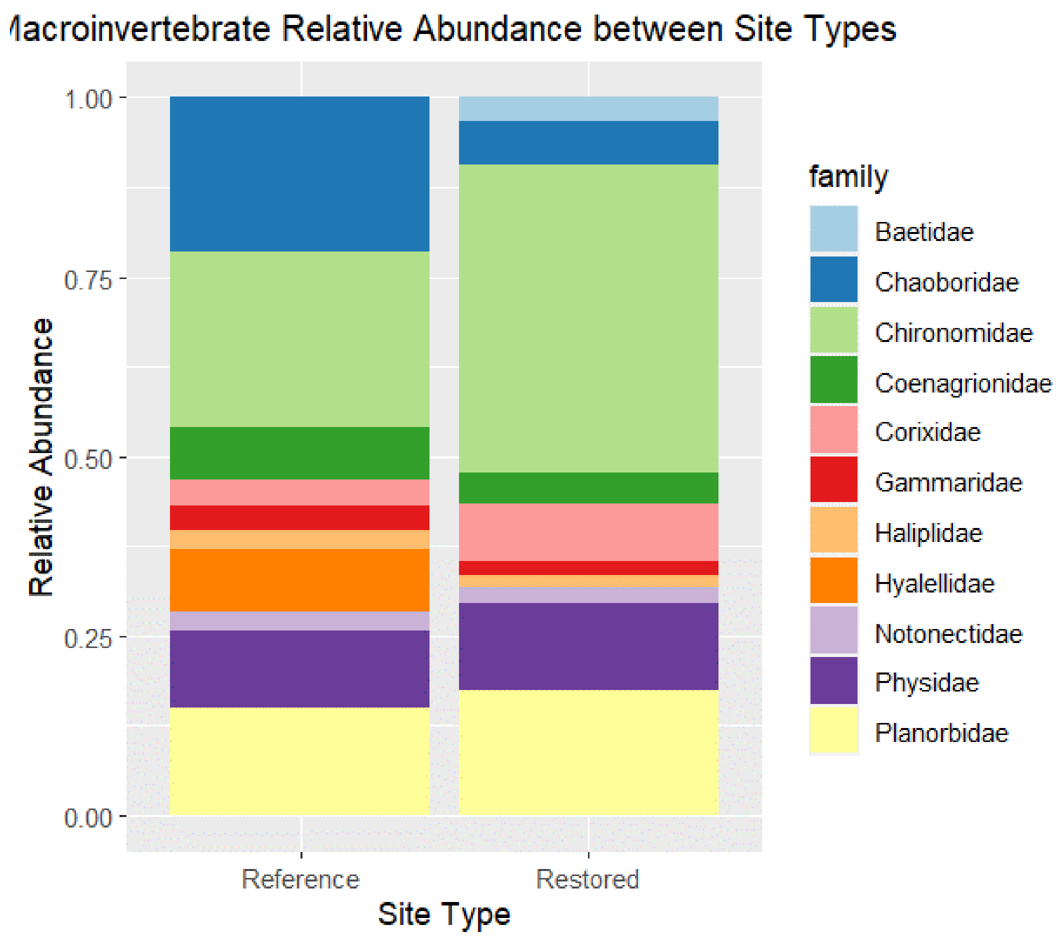

Figure A4. Macroinvertebrate relative abundance based on family-level taxonomy between site types (reference and restored) for all 18 sites.

Table A1. Aquatic macroinvertebrate taxa list from the North Dakota Prairie Pothole Region.

Taxon					Total
Phylum	Class	Order	Family	Genus	(No. of individuals)
Annelida	Clitella	Arhynchobdellida	Erpobdellidae	*Erpobdella*	9
		Rhynchobdellida	Glossiphoniidae	*Glossiphonia*	4
				Helobdella	78
				Theromyzon	1
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	*Galba*	8
				Lymnaea	19
				Stagnicola	173
				Succinea	2
			Physidae	*Aplexa*	18
				Physa	1162
			Planorbidae	*Armiger*	167
				Gyraulus	1342
				Planorbella	37
				Promenetus	129
Arthropoda	Arachnida	Acari	-	-	17
	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	243
			Hyalellidae	*Hyalella*	2522
	Insecta	Trichoptera	Leptoceridae	*Oecetis*	5
			Phryganeidae	*Phyryganea*	1
		Odonata	Aeshnidae	*Anax*	52
			Coenagrionidae	*Enallagma/ Coenagrion*	279
				Nehalennia	261
			Lestidae	*Lestes*	92
			Libellulidae	*Sympetrum*	132
		Hemiptera	Belestomatidae	*Lethocerus*	2
			Corixidae	*Callicorixa*	25
				Cymatia	8
				Hesperocorixa	25
				Palmacorixa	2
				Sigara	86
				Trichocorixa	44
			Gerridae	*Trepobates*	5
			Notonectidae	*Buenoa*	2
				Notonecta	223
			Pleidae	*Neoplea*	5
		Ephemeroptera	Baetidae	*Callibaetis*	230
			Caenidae	*Caenis*	12

Table A1. Aquatic macroinvertebrate taxa list from the North Dakota Prairie Pothole Region (continued).

		Taxon			Total
Phylum	Class	Order	Family	Genus	(No. of individuals)
		Diptera	Ceratopogonidae	*Bezzia*	3
			Chaoboridae	*Chaoborus*	1084
			Chironomidae	-	3730
			Culicidae	*Culex*	23
			Ephydridae	*Setacera*	4
			Muscidae	-	1
			Stratiomyidae	*Odontomyia/ Hedriodiscus*	7
				Stratiomys	1
			Tipulidae	*Prionocera*	1
		Coleoptera	Chrysomelidae	*Donacia*	7
			Curculionidae	-	2
			Dytiscidae	*Agabus*	10
				Coptotomus	3
				Desmopachria	3
				Graphoderus	11
				Hydroporus	1
				Hygrotus	11
				Laccophilus	38
				Laccornis	2
				Liodessus	18
				Rhantus	7
			Haliplidae	*Haliplus*	196
				Peltodytes	3
			Hydrophilidae	*Berosus*	11
				Enochrus	9
				Helophorus	1
				Hydrobius	1
				Laccobius	1
				Tropisternus	10
			Scritidae	-	3