ROLE OF VEGETATED BUFFER ZONES FOR MITIGATING WETLAND PESTICIDE CONTAMINATION AND PROTECTING AQUATIC INVERTEBRATE COMMUNITIES IN NORTHERN PRAIRIE WETLANDS

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ABSTRACT

Prairie Pothole Region (PPR) wetlands are unique resources that provide a number of ecosystem services. However, the majority of these wetlands have been drained or otherwise degraded due to agricultural activities. Wetlands in the PPR are frequently contaminated by agrochemicals from surrounding agriculture, which has been previously demonstrated to have negative impacts on wetland ecology. Vegetation buffers have been proven to be effective in mitigating pesticide and nutrient contamination of water bodies, but have yet to be fully researched in their efficacy in protecting PPR wetlands. Here I examined how multiple agricultural stressors impact PPR wetland health, and whether natural wetland vegetation or producer-implemented perennial plantings are effective buffers, able to mitigate some of the negative effects of agriculture to wetlands. Measurements of pesticides, nutrients, other water quality parameters, in addition to aquatic invertebrate community endpoints were used to comprehensively evaluate the health of PPR wetlands.

Pesticide contamination was widespread, with 59 of the 60 wetlands sampled in 2018 and 2019 containing one or more pesticides in a single growing season. Natural wetland vegetation and the degree of its disturbance from agricultural activities did not have a significant effect on pesticide concentrations in wetlands, although this disturbance did influence the aquatic invertebrate community. Wider and less disturbed wetland vegetation zones were associated with greater macroinvertebrate richness ($p = 0.031$) and greater abundance of Odonata ($p = 0.001$). Aspects of water quality were significant predictors of multiple aquatic invertebrate community indices. The occurrence of cyanobacteria blooms as well as increased total nitrogen (TN) were associated with declines in Shannon's diversity (Cyanobacteria: $p = 0.001$ and TN: $p = 0.016$) and Shannon's Evenness (Cyanobacteria: $p = 0.002$ and TN: $p = 0.001$) as well as increases in Berger-Parker Dominance (Cyanobacteria: $p = 0.004$ and TN: $p = 0.001$). The Pesticide Toxicity Index (PTIs) calculated for each wetland was associated with changes to the aquatic invertebrate community including a decline in total and relative insect abundance ($p = 0.016$ and $p < 0.001$) and an increase in relative snail abundance ($p = 0.005$). Higher PTIs were also associated with a shift in relative abundance of different functional feeding groups ($p = 0.017$). This PTI associated shift in taxa and functional feeding groups likely has greater implications for ecosystem function including the many wildlife species that depend on aquatic insects for food.

Perennial buffers are considered an important management tool to reduce the negative impacts of agriculture on surface waters. Perennial vegetated buffers recently planted under conservation incentive programs were evaluated for their efficacy in mitigating pesticide and nutrient runoff and protecting wetland health. Wetlands that were fully surrounded by perennial buffers and/or other natural vegetation contained significantly lower concentrations of pesticides ($p = 0.001$), lower PTIs ($p < 0.001$), and total phosphorus ($p = 0.005$). However, the presence of perennial buffers alone did not have a significant effect on pesticide or nutrient detections, and even those wetlands that were fully surrounded by perennial buffers or additional natural vegetation all contained some detectable pesticide contamination. The presence of perennial buffers was significantly associated with greater abundances of macroinvertebrates ($p = 0.001$), zooplankton ($p = 0.005$), and insects ($p = 0.039$) which may benefit the many wildlife species that depend on wetland invertebrate productivity for food.

This study establishes a framework for using wetland invertebrate communities as an integrative biomonitoring tool for assessing effects of complex agricultural stressors to PPR wetlands. The results from this study demonstrate negative effects of multiple agricultural stressors on wetland health, as measured by changes in the aquatic invertebrate community. Findings here suggest that leaving or planting wetland vegetation around PPR wetlands could increase community richness and abundance of beneficial insects, but is not sufficient for protecting wetlands from pesticide contamination. However, surrounding wetlands with perennial vegetation plantings in addition to other natural vegetation could be an effective method for reducing pesticide and nutrient contamination of wetlands and increasing the abundance and diversity of aquatic invertebrates, which are an important food source for many wildlife species. These findings may help guide producers and land managers motivated to improve wetland health and ecosystem services in prairie agricultural landscapes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AICc	Akaike information criterion corrected for small sample size
ALUS	Alternative Land Use Services
BMP	best management practice
CABIN	Canadian Aquatic Biomonitoring Network
CAP	Canadian Agricultural Plan
d	Berger-Parker dominance
DCA	detrended correspondence analysis
DUC	Ducks Unlimited Canada
EC50	effective concentration 50
EDM	emergent deep marsh
EH	Shannon's evenness
EMPA	Environmental Management and Protection Act
eq.	equivalent
F	F statistic
GPS	global positioning system
H	Shannon's diversity
ha	hectare
HAB	harmful algal bloom
HBI	Hilsenhoff Biotic Index
HC_5	hazardous concentration protective of 95% of species
IPM	integrated pest management
k	number of estimated parameters in model
km	kilometer
L	liter
LC/MS/MS	liquid chromatography tandem mass spectrometry
LC50	lethal concentration 50
log	logarithm base 10
LOQ	limit of quantification
m	meters
mg	milligram
n	sample size
NGO	non-government organization
NPOC	non-purgeable organic carbon
^oC	degrees Celsius
ON	Ontario
OW	open water
p	probability value
PPR	Prairie Pothole Region

PTI	pesticide toxicity index
R^2	coefficient of determination
RDA	redundancy analysis
RDA1	redundancy analysis axis 1
RDA2	redundancy analysis axis 2
S	richness
SE	standard error
SK	Saskatchewan
SM	shallow marsh
SPEAR	species at risk
SSD	species sensitivity distribution
TDS	total dissolved solids
TEQ	toxic equivalency quotients
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus
TU	toxicity unit
w	AICc weight
WM	wet meadow
ΔAICc	delta AICc
µg	microgram

NOTE TO READERS

This thesis is organized and formatted to follow the University of Saskatchewan College of Graduate and Postdoctoral Studies guidelines for a manuscript-style thesis. Chapter 1 is a general introduction and literature review, including project goals and objectives. Chapter 4 contains a general discussion and overall conclusion. Chapters 2 and 3 of this thesis are organized as manuscripts for publication in peer-reviewed scientific journals. As a result of the manuscript-style format, there is some repetition of material in the introduction and methods sections of this thesis. References cited in each chapter are combined and listed in the 'References' section at the end of the thesis. Supporting information associated with research chapters are presented in the 'Appendix' section at the end of this thesis.

Chapter 1: GENERAL INTRODUCTION

1.1 Prairie Pothole Wetland Hydrology and Ecosystem Services

The Prairie Pothole Region (PPR) is an expansive ecosystem located in the interior of the United States and Canada, encompassing more than 770,000 km^2 (Doherty et al., 2018). Canada's PPR spans three provinces – Alberta, Saskatchewan, and Manitoba, with a landscape defined by the millions of wetlands it contains, referred to as "potholes". These pothole depressions were created when the continental ice sheet retreated at the end of the last glacial period, leaving irregular deposits of moraine and pitted depressions (Pomeroy et al., 2005). Individual PPR wetlands are relatively small. In Saskatchewan, most wetlands do not exceed 0.2 ha in size, and few exceed 10 ha. Despite the small size of most wetlands in Saskatchewan, the cumulative area that PPR wetlands cover is estimated to be 7.3 - 14.2% of the landscape (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). This substantial cumulative land cover is due to the high density of wetlands across much of Saskatchewan's southern half, a density estimated to be 20 wetlands/km^2 on average (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988).

Because the PPR is geologically young, these pothole wetlands remain relatively hydrologically isolated from one another, without an extensive network of streams connecting them to larger bodies of water (Johnson et al., 2008). During spring snowmelt, the majority of runoff collects in wetlands rather than flowing into streams and rivers. Although snow only comprises 25% of precipitation in the PPR, it contributes to 30-50% of the source water runoff collected in PPR wetlands (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). As a result, PPR wetlands often have the greatest basin fill in the spring, and their water levels decrease throughout the summer.

Depending on the basin fill of a wetland and the topography between wetlands, water can flow from one PPR wetland to another. This phenomenon of dynamic connectivity between PPR wetlands is known as "fill and spill" (Shaw et al., 2012). Although runoff collected in PPR wetlands may flow from one wetland to another, the majority of the PPR is considered a non-contributing drainage area. This means that even in years with extremely high precipitation, the water collected in this part of the landscape will never make it to a major river or the ocean (Pomeroy et al., 2005).

The northern Great Plains and the broader PPR are considered to have arid or semi-arid climates. In addition to being far from any ocean, the PPR sits in a rain shadow produced by the

mountains to the west. These two phenomena prevent much of the moisture from the ocean in the form of precipitation from reaching the prairies. Wetlands in the PPR are able to recycle this limited water resource by collecting precipitation and allowing it to evaporate so it can fall once again as rain. Evaporation and transpiration of water to the atmosphere accounts for up to 55% of water losses from PPR wetlands (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). PPR wetlands can recycle precipitation many times over, prolonging the time that water stays in the region. This wetland recycled water can make up a substantial proportion of the precipitation present in summer showers and thunderstorms (Huel, 2000).

In addition to contributing moisture to the atmosphere, PPR wetlands are able to contribute water to aquifers through groundwater recharge (van der Kamp & Hayashi, 1998). In fact, smaller, more ephemeral wetlands have been shown to contribute more to groundwater stores than wetlands with more permanent water bodies (LaBaugh et al., 1998). The ability of PPR wetlands to store and recycle water allows them to act as a reservoir in years of drought. In permanent and semi-permanent wetlands, collected runoff can remain in the wetland basin during subsequent years and moderate some of the effects of drought in the surrounding area (Woo & Rowsell, 1993). Wetlands can also reduce the risk of flooding after intense runoff events by capturing water in their basins.

The ecosystem services of PPR wetlands are not limited to their ability to regulate water. These wetlands have also been demonstrated to be effective in their ability mitigate negative impacts of soil erosion by collecting soil/sediment that could otherwise end up downstream (Pomeroy et al., 2005). By retaining sediment and other residues during runoff, wetlands improve downstream water quality, trapping excess nutrients an providing a habitat for microbes that process these nutrients (Johnston, 1991). The natural biochemical processes occurring in wetlands can also sequester carbon in wetland soils and sediments, helping offset greenhouse gas emissions. After accounting for methane produced by PPR wetlands, researchers in one study found that restored PPR wetlands have the potential to sequester approximately 3.25 million grams CO_2 eq. ha^{-1} $year^{-1}$ (Badiou et al., 2011).

As important as PPR wetlands are for regulating water and improving water quality, these wetlands also support very high levels of biodiversity. In addition to benefitting from improved water quality and water regulation, many wildlife species seasonally depend on intact wetlands

for habitat. For example, over 150 species of birds and other animals use Saskatchewan's PPR wetlands for habitat (Huel, 2000). Over half of the ducks surveyed annually in North America breed in or around PPR wetlands (Janke et al., 2017). In addition to many resident species, large numbers of migratory birds use PPR wetlands as essential stopover sites during migration (Janke et al., 2019). Many migratory shorebirds, some of which have conservation status as endangered or threatened (Morrison et al., 2001; National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988), are found in large numbers in Prairie wetlands during their spring and fall migrations. Wetlands also provide habitat for native bees and other pollinators to nest and forage (Vickruck et al., 2019). While most PPR wetlands do not hold commercial fish, they do support diverse and productive communities of aquatic invertebrates and zooplankton communities which are relied upon by many vertebrates as food (Euliss et al., 1999). Consequently, PPR wetlands are highly productive biodiversity hotspots in an otherwise depauperate agriculture dominated landscape.

1.2 Prairie Pothole Wetland Conservation Challenges

The prairies of Canada account for approximately 85% of the total arable land in the entire country (Campbell et al., 2011). Often known as the "bread basket" of Canada, this region produces much of the country's cereals, grains, and other annual crops such as oil seeds and pulses. In 2020, Saskatchewan alone produced 16 million tonnes of wheat on 12.8 million acres of land and 10.2 million tonnes of canola seed on 11.3 million acres (Statistics Canada, 2020).

Due to the intensive agriculture that takes place in the PPR, it is considered one of the most highly altered landscapes in the world. Many wetlands in this region have been drained to increase land for crop production. Estimates indicate that before the prairies were settled, wetlands comprised more than 20% of total PPR land area, while today up to 89% of PPR wetlands have been lost due to agricultural drainage (Doherty et al., 2018). In 1970, it was estimated that roughly 1.2 million hectares of Canada's PPR wetlands had been converted to agricultural use (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988) with losses continuing over the past 50 years.

Many of the remaining wetlands in the PPR that evaded being drained are still physically impacted by agricultural activities. Wetland margins, which represent the transition area between the open water and the drier upland zones, are frequently manipulated or disturbed by tilling,

burning, herbicide spraying, trampling, and grazing. In 2005, 95% of the remaining PPR wetlands in Saskatchewan were found to have their margins altered or degraded due to agriculture (Bartzen et al., 2010). Clearing wetland margin vegetation (e.g., cattails) can decrease wetland size and depth by reducing winter snow trapping and accumulation. Removal of vegetation from margins can also promote increased erosion and eventual filling of the wetland basin with silt (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). Currently there is a lack of protection to prevent wetland degradation in Canada.

1.3 Pesticide Contamination of PPR Wetlands

In addition to being physically altered by agricultural activities, many of the remaining PPR wetlands are also impacted by agriculture through pesticide and fertilizer contamination. Despite the fact that the region is the largest user of agrochemicals in Canada (Kissinger & Rees, 2009), pesticide contamination of wetlands has historically been poorly characterized in Canada's prairies. In a 1997 Agriculture and Agri-Food Canada report on the effects of agriculture on water quality in Canada's prairies; the authors concluded that "no evidence was presented of widespread, long-term agricultural pollution in excess of Canadian Water Quality Guidelines" (Brook Harker, 1997). A more comprehensive study was performed by Environment Canada, assessing pesticide contamination of Saskatchewan's PPR wetlands between 1991 and 1996. This study found as many as 6 pesticides in a single wetland and 24% of wetlands to have levels of pesticides in exceedance of Water Quality Guidelines in a single year (Donald et al., 1999). The insecticide lindane and the herbicide triallate were found to exceed guidelines more than any of the other pesticides detected. This study also found that pesticides increased in frequency and concentration as precipitation increased regionally (Donald et al., 1999).

While lindane and other problematic chemicals have since been banned for use in Canadian agriculture, pesticide use has evolved and continued to increase over time (Malaj et al. 2020) . For example, in the 1990s, neonicotinoids were introduced and are now the most widely used class of insecticide in the world (Wood & Goulson, 2017). The use of neonicotinoids as seed treatments has led to the rapid increase in neonicotinoid use as a prophylactic pest management strategy (Douglas & Tooker, 2015). Today, seeds for many of the cash crops of North America including corn, soybean, and canola are sold with neonicotinoid seed treatments, with few

commercially available alternatives (Simon-Delso et al., 2015; Wood & Goulson, 2017). In the PPR, neonicotinoids have become the principal seed treatment for control of canola pests such as flea beetles (Coleoptera: Chrysomelidae). Neonicotinoid treated canola seeds are used on over 95% of the canola planted in Canada annually (Soroka et al., 2018), with 229 tonnes of insecticide applied as canola seed treatments in the PPR in 2015 (Malaj et al., 2020). Given this massive increase in neonicotinoid prophylactic use in seed treatments as well as their high environmental persistence and water solubility (Goulson, 2013), neonicotinoids have been frequently detected in surface waters including wetlands throughout the PPR of Canada. In a study investigating neonicotinoid contamination of PPR wetlands in Saskatchewan, researchers found that 91% of the 90 PPR wetlands sampled in spring 2013 were contaminated with one or more neonicotinoids (Main et al., 2014).

1.4 Aquatic Invertebrates as Bioindicators and Their Response to Agricultural Stressors

Invertebrates are used extensively as bioindicators of ecosystem health. The widespread abundance and diversity of invertebrates across diverse environments allow for their use as bioindicators in many different contexts. A relatively small study area can contain large numbers of invertebrate species and individuals. This allows for species, population, and community level analyses. Invertebrates also generally have faster growth and population turn-over rates than larger animals, making invertebrates more responsive indicators of environmental changes (Hodkinson & Jackson, 2005). The utility of aquatic invertebrates as bioindicators is evident in their well-established use in assessment of water quality and ecosystem health of fresh water streams (Gaufin, 1973; Lenat, 1988; Metcalfe, 1989).

Pesticide contamination of streams can exert differential toxicity to specific invertebrate species and thereby alter aquatic invertebrate community composition (Beketov et al., 2013; Berenzen et al., 2005; Liess et al., 2008; Liess & von der Ohe, 2005). Liess and von der Ohe (2005) used a species at risk (SPEAR) approach, which classifies invertebrate taxa based on a set of sensitive traits that increases their risk of experiencing adverse effects. In this study, pesticide concentrations were converted to cumulative toxicity units (TUs), allowing researchers to assess the effects of pesticide mixtures. Liess and von der Ohe (2005) found that pesticide concentrations $1/10^{th}$ the acute 48-hour median lethal concentrations (LC50s) for *Daphnia*

magna caused short- and long-term reductions of SPEAR in the 20 streams studied. Concentrations 1/100th the acute 48-hour LC50s for *D. magna* caused long term changes in aquatic invertebrate community composition.

Different methods for assessing the effects of pesticide contamination on stream aquatic invertebrate communities have produced similar findings. For example, Berenzen et al. (2005) used multivariate statistical modeling to interpret macroinvertebrate taxa abundance data. In a canonical correspondence analysis constrained by environmental variables and pesticide TUs, the researchers found that streams with higher pesticide TUs had distinct differences in community composition compared to control sites. A redundancy analysis (RDA) of the multivariate invertebrate taxa abundances explained 95% of variance, with pesticide TU being the only significant constraining variable. The total pesticide concentrations of the streams exposed to pesticides were between 1/5th and 1/100th of the acute 48-hour LC50s for *D. magna*.

Yet another method of interpreting aquatic invertebrate community data is to convert invertebrate taxa abundances to community indices. Beketov et al. (2013) used aquatic invertebrate abundances to calculate richness and other diversity indices for streams with varying levels of pesticide contamination. Researchers found a 45% decrease in taxonomic richness in highly contaminated streams compared to uncontaminated streams. Effects on taxa richness were found in streams with pesticide TUs ranging from 1/10,000th to 1/100th of the pesticide EC50s for *D. magna*.

Collectively, these three stream studies evaluating the effects of pesticide contamination on aquatic invertebrate communities all found adverse effects of pesticides at concentrations below what might be expected considering toxicological benchmarks based on a single species, *D. magna*. One study investigating the toxicity of organic compounds on a range of different aquatic invertebrates found that 22% of the investigated taxa were more sensitive to organic compounds than *D. magna* (von der Ohe & Liess, 2004). These more sensitive taxa included Plecoptera, Amphipoda, and some non-*D. magna* Cladocera. Pesticide toxicity to more sensitive taxa such as those identified by von der Ohe & Liess (2004) could explain the community level effects of low concentrations of pesticides observed in stream ecosystems. In addition, a study using in-situ aquatic enclosures (limnocorrals) in wetlands was able to expose aquatic invertebrate communities to mixtures of neonicotinoids, finding that the negative effects of neonicotinoids on insect emergence occurred at concentrations below that observed from lab-

derived model predictions (Maloney et al., 2018). This highlights the need to consider toxicological effects at a community level based on aquatic organisms with varying sensitivities in order to evaluate and set water quality criteria that are more protective of both sensitive species and overall community composition. These studies also demonstrate the importance of field studies for investigating ecosystem effects, as opposed to solely applying lab-based benchmarks derived from individual species.

While lab studies allow researchers to carry out controlled exposures of individual chemicals to specific study organisms, real environmental scenarios often involve complex mixtures of multiple contaminants. A Pesticide Toxicity Index (PTI) is a tool developed to summarize the predicted toxicity of a pesticide mixture based on concentrations of each mixture component relative to toxicity values from multiple species compiled into a species sensitivity distribution (SSD) (Nowell et al., 2014). By contrast, TUs are often based off of just one species such as *D. magna* (Beketov et al., 2013; Berenzen et al., 2005; Liess & von der Ohe, 2005). Therefore, PTIs could serve as a more accurate predictor of pesticide mixture toxicity to aquatic ecosystems.

Studies utilizing aquatic invertebrates for biomonitoring have largely overlooked PPR wetlands, with the majority of these studies focused on streams and rivers. While a PTI approach to investigate the effects of pesticide mixtures has yet to be conducted on PPR wetlands, there is evidence that pesticides mixtures are having negative impacts on wetland aquatic invertebrates (Cavallaro et al., 2018, 2019; Maloney et al., 2018; Schepker et al., 2020). In a previous study, limnocorrals were dosed with field-relevant concentrations of neonicotinoids to study the effects of these pesticides on aquatic insect emergence. Analysis of insect taxa abundance captured in emergence traps revealed that limnocorrals dosed with imidacloprid and clothianidin had significantly lower abundance of multiple chironomid species than what was measured in control limnocorrals. Interestingly, in the limnocorrals dosed with imidacloprid and clothianidin, chironomid and zygopteran emergence was advanced by 18 to 25 days compared to controls (Cavallaro et al., 2018). These findings demonstrate that neonicotinoid contamination could be altering community dynamics and phenology of aquatic emergent insects in PPR wetlands. A similar study was performed in which researchers examined the abundance of aquatic emergent insects in PPR wetlands receiving neonicotinoid pesticide contamination from surrounding fields. Toxic equivalency quotients (TEQs) were calculated for each wetland using neonicotinoid

concentrations measured in wetlands as well as chironomid chronic toxicity values. In an RDA of emergent aquatic insect abundance constrained by neonicotinoid TEQs and other environmental variables, neonicotinoid TEQs were significant in their effect on emergent aquatic insect abundances (Cavallaro et al., 2019).

Cavallaro et al. (2018, 2019) demonstrated that environmentally relevant concentrations of neonicotinoids could reduce emergence of specific aquatic insect taxa, impacting the relative abundances of certain taxa within the emergent insect community. However, these studies are limited to those taxa that are captured in emergence traps. While emergent insects represent a significant portion of aquatic invertebrates found in wetlands, much of the wetland invertebrate community is made up of non-insect and non-emergent invertebrate species that are not effectively captured in emergence traps such as Amphipoda, Gastropoda, Corixidae, and many aquatic Coleoptera. Schepker et al. (2020) sampled aquatic invertebrates in Nebraska wetlands with an aquatic sweep net, using methods similar to those used in aquatic biomonitoring of streams. The authors found that the concentration of neonicotinoids detected in wetlands was significantly associated with declines in aquatic invertebrate biomass. While these studies provide evidence that neonicotinoids may be affecting emergent aquatic taxa, there is a lack of knowledge of how the entire aquatic invertebrate community may be impacted by multiple pesticide contamination (beyond neonicotinoids) in PPR wetlands.

1.5 Value of Vegetated Buffer Implementation

Given the unique hydrology and recognized importance of PPR wetlands for their ecosystem services (Badiou et al., 2011; Huel, 2000; Janke et al., 2017, 2019; Johnston, 1991; LaBaugh et al., 1998; Morrison et al., 2001; National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988; Pomeroy et al., 2005; van der Kamp & Hayashi, 1998; Vickruck et al., 2019; Woo & Rowsell, 1993) as well as increasing threats to wetland ecosystems due to agriculture (Bartzen et al., 2010; Doherty et al., 2018; Donald et al., 1999; Main et al., 2014; National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988), there has been some effort and public interest to improve wetland protection using vegetated buffer zones between agricultural fields and water bodies. However, most of the literature on the efficacy of vegetated buffers has been focused on protecting streams and rivers, with very little research aimed at wetlands, and even less on PPR wetlands.

1.5.1 Reduction of Pesticide and Nutrient Runoff by Vegetated Buffers

Implementing vegetated buffers is often promoted as a mechanism to reduce surface water contamination with pesticides and other harmful chemicals like excess nutrients. While many field studies have investigated the efficacy of vegetated buffers to mitigate pesticide and nutrient runoff, the implementation of vegetated buffers and the environmental conditions in which they exist are highly variable making interpretation and generalizations difficult. To better understand the findings that have been produced from this body of work, Prosser et al. (2020) conducted a review of studies assessing the effect of vegetated buffers on pesticide and/or nutrient contamination of waterbodies. They found a strong consensus that vegetated buffers did indeed reduce pesticide and nutrient loads, although the degree to which these contaminants were reduced was extremely variable. Pesticide reductions from vegetated buffers ranged from 10% to 100% and nutrient reduction ranged from 12% to 100%. While many aspects of vegetated buffers were assessed, the most frequently tested parameter was buffer width. Across all the studies investigating the efficacy of vegetated buffers, analysis suggested that generally larger buffer widths does increase the capacity of vegetated buffers to reduce pesticides and nutrients in runoff. However, there is no consensus on what is the minimum or optimal width of vegetated buffer for mitigating contaminant transport, while considering that this area requires taking agricultural land out of production (Prosser et al., 2020).

1.5.2 Increase in Biodiversity and Agricultural Productivity by Vegetated Buffers

In addition to reducing pesticide and nutrient contamination in water bodies, vegetated buffers can increase the overall biodiversity of agroecosystems. One study found that implementing vegetation strips in corn and soy fields increased bird species richness and abundance by factors of 2.1 and 2.6, respectively, and increased the number of insect taxa found in crop fields by a factor of 2.6 (Schulte et al., 2017). Terrestrial insect biodiversity provides valuable ecosystem services to agriculture. Many native insects and honey bees provide pollination services which can increase the yield of crops including canola (Sharma & Reddy, 2020). Outbreaks of pest insects can be avoided or delayed by the presence of predatory and parasitic insects and spiders which consume pest insects (Jones & Snyder, 2018). Insectivorous birds have been found to preferentially forage around wetlands in PPR agricultural landscapes

(Elgin et al., 2020), making vegetated buffers and the insect populations they support a potential added resource for this wildlife.

Although implementation of vegetated buffers often requires the conversion of cropland to non-crop area, the remaining cropland has been shown to have higher crop yields in some cases. Schulte et al. (2017) observed significantly greater corn yield on cropland of fields with vegetated buffers. Additionally, once vegetated buffers are established, there are little to no costs associated with their maintenance. So although land converted to vegetated buffer does not produce any crop, it has the potential to benefit adjacent cropland without requiring the monetary inputs needed to produce annual crops (annual seed and agrochemical expenses) (Schulte et al., 2017). It is also possible for producers to use buffer areas for hay or livestock forage, creating additional value of these areas (Huel, 2000).

1.5.3 Planting of Vegetated Buffers Around Wetlands

Relatively few studies have explored the efficacy of vegetated buffers around wetlands (Aguiar et al., 2015; Moore et al., 2014; Schepker et al., 2020), and even fewer have examined vegetated buffers around PPR wetlands (Cavallaro et al., 2019; Main et al., 2015). Studies conducted on non-PPR wetlands have demonstrated the ability of vegetated buffers to reduce pesticide (Moore et al., 2014) and nutrient (Aguiar et al., 2015) runoff into wetlands. Another study found that concentrations of neonicotinoids in wetlands were lower in those surrounded by 50 m or more of non-crop vegetation between the wetland water and cropland (Schepker et al., 2020).

A study conducted on PPR wetlands in central Saskatchewan found that wetlands with more diverse and intact natural wetland vegetation around them were less likely to be contaminated with neonicotinoids from surrounding agriculture (Main et al., 2015). Notably, the plant composition of the natural wetland zones (ie. the shallow marsh zone) had a larger effect on neonicotinoid contamination than buffer width alone. Reduced disturbance of natural vegetation around PPR wetlands from agriculture can positively impact aquatic invertebrate communities in wetlands. Wetlands surrounded by continuous vegetation were found to have greater diversity of aquatic insects caught in emergence traps (Cavallaro et al., 2019).

These studies conducted on PPR wetlands provide some evidence that natural vegetation presence and composition can effectively mitigate specific pesticide contamination of wetlands

and may have a benefit to aquatic invertebrate communities. However, each of these studies has focused on characterizing natural vegetation as a wetland buffer (Cavallaro et al., 2019; Main et al., 2015). There are no studies explicitly testing the efficacy of perennial vegetation plantings around PPR wetlands to understand if they can similarly mitigate or reduce pesticide contamination.

1.5.4 Programs Incentivizing Wetland Protection Through Implementation of Vegetated Buffers

Only wetlands on federal land fall under the jurisdiction of the Government of Canada, leaving most wetlands in Canada under the control of provincial governments or private landowners. The province of Saskatchewan adopted the Environmental Management and Protection Act (EMPA) in 2000, protecting wetlands on Crown land and requiring a permit for development of these areas. However, the EMPA does not provide any protection for wetlands on privately owned land (Rubec & Hanson, 2009). The Saskatchewan Water Security Agency requires landowners to obtain a permit to drain wetlands off their land, but this rule is difficult to enforce and as a result, many landowners drain and degrade wetlands with little oversight.

Despite a lack of scientific evidence, implementing or leaving vegetated buffers around wetlands has been a recommended best management practice (BMP). In *Managing Saskatchewan Wetlands, a Landowner's Guide (2000)*, the recommended minimum buffer is 10 m of perennial vegetation between the crop and the edge of a wetland, not including the area of natural wetland vegetation which is itself part of the wetland basin. This is problematic as it is often only the wetted area or open water that producers regard as the wetland. Fluctuations in water levels between and within years makes it a challenge to manage wetland margins when adjacent crops are under intensive production. Huel (2000) also recommends leaving a wider vegetation buffer around wetlands in areas where soil is prone to erosion or where there are issues of salinity.

Several non-government organizations (NGOs), such as Ducks Unlimited Canada (DUC) and Alternative Land Use Services Canada (ALUS) are actively seeking solutions to better conserve and protect wetland habitat in the PPR. In addition, the provincial governments will provide support under the Canadian Agricultural Plan (CAP) to recover some costs for perennial plantings near wetlands as a strategy to incentivize their protection. DUC has partnered with

government agencies and private landowners to find solutions to reduce wetland degradation and loss. Providing landowners with resources and incentive programs, NGOs can offer these landowners benefits for protecting wetlands on their property. DUC has aided in more than 11,890 wetland projects, securing 6.4 million acres of wetland habitat (Ducks Unlimited Canada, 2019). Similarly ALUS has partnered with thousands of agricultural producers to find solutions to soil erosion, nutrient loading, and degradation of wetlands in Canada (ALUS Canada, 2019). While these incentive programs exist for Saskatchewan producers to convert cropland to perennial forage in areas around wetlands on their fields, there is a lack of understanding in how effective these strategies are for achieving goals to improve wetland health.

1.6 Research Objectives

The overarching goal of this thesis is to assess the impacts that agriculture is having on PPR wetland heath and evaluate perennial vegetation management actions as a strategy to minimize negative effects of pesticides, nutrient, and wetland habitat degradation. My first objective is to determine how aquatic invertebrate community indicators of wetland health are being impacted by agriculture using multiple metrics of vegetation disturbance, pesticide contamination, nutrient and other water quality parameters.

I address this first objective in Chapter 2, where I present a study of PPR wetlands within actively farmed fields in Saskatchewan to examine natural wetland vegetation disturbance, pesticide contamination, nutrient and water quality parameters, and relate these to the aquatic invertebrate taxa abundances and several commonly used community metrics. First, I assessed if there was a relationship between natural wetland vegetation disturbance and concentrations of pesticides and nutrients, and on other water quality parameters measured in wetlands. I hypothesized that higher degrees of vegetation disturbance would be associated with higher pesticide and nutrient concentrations in wetlands. Second, I evaluated which factors of wetland vegetation disturbance, pesticide contamination, nutrient concentrations, water quality parameters, and other environmental features were most strongly associated with changes in the aquatic invertebrate community (e.g., abundance and biotic indices such as diversity and richness). To evaluate the potential effects of pesticide mixtures on the aquatic invertebrate community, I calculated PTIs for each wetland based on concentrations of pesticides measured in water relative to a calculated HC_5 value from Species Sensitivity Distributions of invertebrate

toxicity values. I hypothesized that several agricultural stressors would significantly impact the aquatic invertebrate community, with increasing PTI associated with lower diversity and richness of aquatic invertebrates.

My second objective is to evaluate the effectiveness of perennial vegetated buffers implemented through DUC and ALUS programs on private farms to improve wetland health. To address this second objective in Chapter 3, I present a study examining PPR wetlands within actively farmed fields with previously planted and established DUC or ALUS perennial vegetation buffers. Aspects of perennial buffer configurations around wetlands, along with pesticide contamination (PTI), nutrient and other water quality parameters, and aquatic invertebrate taxa abundances were measured in each study wetland. First, I tested whether perennial buffers and their different configurations reduced pesticide and nutrient concentrations measured in wetland water bodies. I hypothesized that the presence of perennial buffers as well as a higher degree to which wetlands are surrounded by perennial buffers would be associated with lower pesticide and nutrient concentrations. Second, I modelled which factors of perennial buffers, pesticide contamination, nutrient and water quality parameters, and environmental factors influenced the aquatic invertebrate community (e.g., abundance and biotic indices such as diversity and richness). I hypothesized that wetlands surrounded by perennial buffers would have greater diversity and richness in their aquatic invertebrate communities compared to wetlands that are not surrounded by perennial buffers.

This thesis creates a framework for assessing PPR wetland health using aquatic invertebrate biomonitoring, a tool that has not been widely applied to this aquatic ecosystem. This framework is then used to assess the effects of agricultural stressors on PPR wetlands, and to identify the relative role of vegetated buffers in altering aspects of wetland health, including the aquatic invertebrate community. I anticipate that the findings from this work will aid other efforts to assess the health of PPR wetlands.

Chapter 2: QUANTIFYING THE EFFICACY OF NATURAL WETLAND VEGETATION AS BUFFERS FOR MITIGATING AGROCHEMICAL CONTAMINATION AND PROTECTING AQUATIC INVERTEBRATE COMMUNITIES

2.1 Abstract

The Prairie Pothole Region (PPR) wetlands are a unique resource that provides a number of ecosystem services. However, the majority of these wetlands have been drained or otherwise physically and chemically degraded due to agricultural activities. Wetlands in the PPR are frequently contaminated by pesticides from surrounding agriculture, which has been previously demonstrated to have negative impacts on wetland ecology. Here we examine how agricultural stressors impact PPR wetland health, and whether natural vegetation surrounding wetlands can serve as a buffer, mitigating some of these negative effects to wetlands. Concentrations of pesticides, nutrients, other water quality parameters, and aquatic invertebrate community composition endpoints were used to evaluate the health of PPR wetlands in linear models and multivariate analyses. Aquatic invertebrate community composition endpoints used in this study included biotic indices, abundance of certain invertebrate taxa, and relative abundance of certain invertebrate taxa (abundance of an invertebrate taxa relative to total macroinvertebrate abundance). In the 34 wetlands sampled in the 2018 growing season, 24 different pesticides were detected, with quantifiable concentrations of pesticides measured in each wetland. Neonicotinoids were the most frequently detected pesticide class, detected in 74% of water samples. Natural wetland vegetation disturbance was not found affect the concentrations of pesticides measured in wetlands. However, greater width of natural vegetation zones left between cropland and wetland water bodies was significantly associated with a shift in multivariate water quality parameters ($p = 0.001$) as well as an increase in macroinvertebrate richness ($p = 0.031$). Additionally, vegetation disturbance in the form of loss of percent vegetation cover was significantly associated with a decline in total and relative abundance of Odonata ($p = 0.001$ and $p = 0.001$).

Water quality parameters were also significant predictors of multiple aspects of wetland aquatic invertebrate communities. The occurrence of cyanobacteria blooms as well as increased total nitrogen (TN) were associated with declines in Shannon's diversity (Cyanobacteria: $p < 0.001$ and TN: $p = 0.016$), increases in Berger-Parker Dominance (Cyanobacteria: $p = 0.004$ and TN: $p < 0.001$), and multiple other changes in aquatic invertebrate communities. Pesticide Toxicity Indices (PTIs) calculated from concentrations of pesticides detected in wetlands relative to their respective published toxicity values were also associated with changes in the aquatic invertebrate community including a decline in total and relative insect abundance ($p = 0.016$ and

$p < 0.001$) and an increase in relative snail abundance ($p = 0.005$). Higher PTIs were also associated with a shift in relative functional feeding group abundance ($p = 0.007$) in a partial redundancy analysis. This observed PTI associated shift in taxa and feeding group abundance likely has implications for ecosystem function. Reduced abundance of aquatic insects could have serious negative impacts on the many wildlife species that depend on wetland insects for food. The findings presented here demonstrate the adverse effects of multiple agricultural stressors on PPR wetland health. This study also provides a framework for using aquatic invertebrate communities sampled using the new CABIN protocol as an integrative biomonitoring tool for assessing the effects of complex agricultural stressors on wetland ecosystems.

2.2 Introduction

Wetlands in Canada's PPR are hydrologically unique features that provide many ecosystem services to humans and the environment including groundwater recharge (LaBaugh et al., 1998), flood and drought mitigation (Huel, 2000; Woo & Rowsell, 1993), water purification (Johnston, 1991; Pomeroy et al., 2005), carbon sequestration (Badiou et al., 2011), and support of biodiversity (Doherty et al., 2018; Huel, 2000; Janke et al., 2017, 2019; Morrison et al., 2001; National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). Due to intensive agricultural activity on the prairies, up to 89% of PPR wetlands have already been lost due to drainage (Doherty et al., 2018), and 95% of the remaining PPR wetlands in Saskatchewan have their margins physically disturbed or manipulated due to agriculture (Bartzen et al., 2010). Wetland margins typically consist of the outer vegetation zones that contain specific vegetation reflective of the duration and frequency of inundation in that zone (Huel, 2000).

In addition to the physical impacts agriculture can have on wetlands through wetland drainage or degrading plant communities in wetland margins, agricultural chemical inputs can also negatively affect water quality. Agricultural activities in the PPR affect nutrient and water quality dynamics (Detenbeck et al., 2002) and runoff from surrounding farmland frequently contaminates wetlands with neonicotinoid insecticides (Main et al., 2014). Large expanses of the prairie region are at high risk of pesticide contamination based on recent modelling (Malaj et al., 2020).

Several pesticides are widely used and represent significant concern for wetland invertebrate communities. For example, in 2015, 229 tonnes of insecticide were applied as canola seed treatments primarily consisting of neonicotinoids, in the PPR of Canada (Malaj et al., 2020). The neonicotinoids are prophylactically used in seed treatments of many dominant prairie crops including canola, cereals, and pulses, leading to widespread use and environmental contamination from these insecticides. Pesticide contamination of PPR wetlands, specifically by neonicotinoid insecticides, has been linked to shifts in emergent aquatic insect community dynamics and reduced abundance of emerging insects (Cavallaro et al., 2018, 2019). Nutrients and water quality of wetlands such as conductivity and total nitrogen are strong predictors of aquatic invertebrate community composition (Spieles & Mitsch, 2000). Additionally, physical disturbances to wetlands through vegetation removal have been linked to lower emergent insect diversity (Cavallaro et al., 2019). Aquatic invertebrates have long been used for biomonitoring of aquatic lotic and lentic systems (Hodkinson & Jackson, 2005), yet relatively few studies have been conducted on wetland ecosystems.

One potential solution to mitigate the damaging effects of agricultural disturbance and resultant pesticide and nutrient contamination in our PPR wetlands is for producers to retain intact natural wetland vegetation zones. Previous studies have found that intact shallow marsh wetland vegetation is linked with reduced neonicotinoid contamination in PPR wetlands (Main et al., 2015). Planting vegetated buffers in other agricultural settings is effective in reducing pesticide and nutrient contamination of water bodies (Prosser et al., 2020) while also increasing agroecosystem biodiversity (Schulte et al., 2017).

Aquatic invertebrate biomonitoring in prairie wetlands could prove to be an effective integrative tool for assessing multiple agricultural stressors and different management solutions. Adapting the approach of aquatic invertebrate biomonitoring developed for streams, we used the CABIN protocol for wetlands (Environment and Climate Change Canada, 2018) alongside multiple community biomonitoring metrics to understand how agricultural stressors of pesticides, nutrients, and physical vegetation disturbance affect the health and condition of poorly studied PPR wetland ecosystems.

The objectives of this study were to a) examine the relationships between natural wetland vegetation and its disturbance on pesticide and nutrient concentrations in PPR wetlands of Saskatchewan and b) determine which aquatic invertebrate community metrics are most sensitive

to agricultural disturbance and may therefore be used as indicators of wetland health. I hypothesized that increased disturbance of natural wetland vegetation would be associated with greater pesticide and nutrient concentrations in wetlands, as well as significant changes in aquatic invertebrate community structure. Additionally, I hypothesized that increasing levels of pesticide contamination would result in lower aquatic invertebrate richness and diversity.

2.3 Methods

2.3.1 Study Site Selection

In 2018, 34 fishless wetlands located on privately owned cropland were selected within 180 km east of Saskatoon, Saskatchewan. A number of these study wetlands were located on the same field or adjacent fields, within 15 km of one another, allowing the wetlands to be grouped into six geographic clusters or "blocks" (Figure 2.1). Wetlands were primarily surrounded by plantings of either canola or wheat annual crops, except for three wetlands located on fields planted with field peas or corn. While all study wetlands were on actively cultivated fields, some were located along field margins or adjacent to other natural vegetation areas. As a result, a portion of the perimeter of these wetlands was not directly adjacent to or surrounded by crop (Figure 2.2).

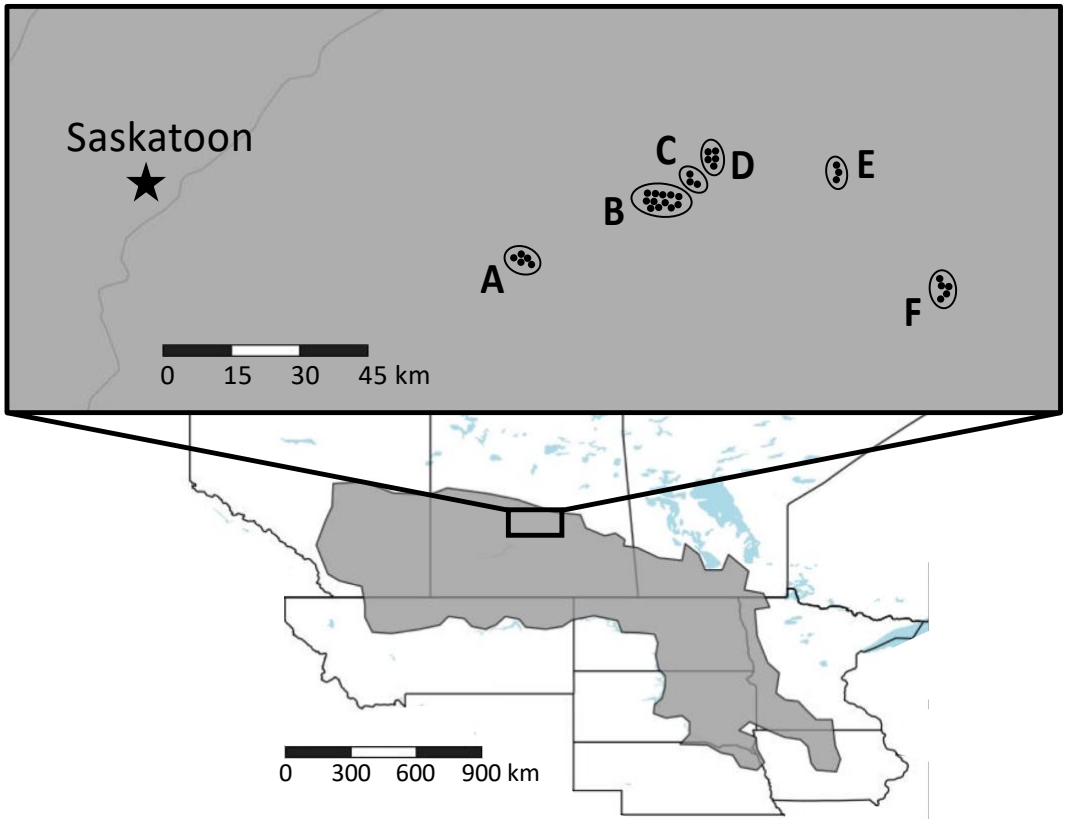

Figure 2.1. Map of wetland study sites grouped into regional "blocks" based on geographic proximity to one another. Wetlands are represented as black dots within blocks represented as black ovals. All sites were located in the northern Canadian PPR (grey shaded area). The 6 blocks containing 34 wetland sites include block A with 5 wetlands, block B with 12 wetlands, block C with 3 wetlands, block D with 5 wetlands, block E with 3 wetlands, and block F with 5 wetlands.

2.3.2 Quantification of Wetland Disturbance

As a result of previous and ongoing agricultural activities around PPR wetlands, natural vegetation in the wetland vegetation zones is often physically disturbed or removed. This can be a result of tillage and herbicide spraying around the wetland basin. The degree of natural vegetation disturbance and removal can be affected by individual farming practices in addition to other environmental factors. As a result, each study wetland varied in the degree to which its vegetative composition was disturbed or removed.

Natural vegetation disturbance was quantified along the cropped perimeter of study wetlands. For each wetland, the percentage of the wetland perimeter surrounded by crop was measured (percent crop). This cropped perimeter of each wetland was further characterized,

measuring multiple aspects of wetland disturbance (zone width and vegetation disturbance) within the impacted area. The remaining proportion of the wetland perimeter that was surrounded by natural non-crop area (percent non-crop) such as a grassy field margin was recorded but not further characterized (Figure 2.2).

The first aspect of vegetation disturbance measured in wetlands, zone width, quantifies the distance of natural wetland vegetation zone remaining between the cropland and the open water. This area consisted of the three wetland vegetation zones of (the emergent deep marsh (EDM), shallow marsh (SM), and wet meadow (WM)) which were identified based on plant communities known to grow in each zone (Stewart & Kantrud, 1971). The combined width of these three zones was measured along four transects in each wetland (Figure 2.2). Zone width was only measured along transects that terminated along the cropped edge of wetlands. Average vegetation zone width (zone width) was calculated for each wetland as the sum of the zone widths measured along each transect divided by the number of transects measured.

Vegetation disturbance (percent absence of vegetation cover) was also measured in each of the three wetland vegetation zones along the same transects used to measure zone width (Figure 2.2). Along each transect, percent cover of wetland vegetation was measured by zone, based on assessing the area one meter to the left and right of the transect. Vegetation cover measured in each zone was then averaged across the four transects for each zone (average vegetation cover). Then average percent vegetation disturbance across the three zones (vegetation disturbance) was calculated using the equation below.

$$\textit{Vegetation Disturbance} = 1 - \sum_{n=1}^{3} \frac{\boldsymbol{average\ vegetation\ cover\ of\ zone}}{\mathbf{3}} \qquad \text{(Eqn. 2.1)}$$

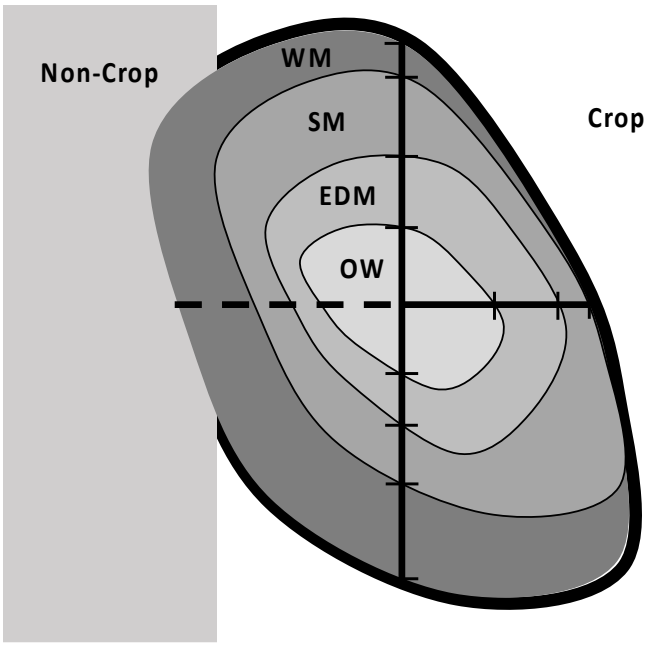

Figure 2.2. Diagram of measurements taken at each study wetland. The wetland zones wet meadow (WM), shallow marsh (SM), and emergent deep marsh (EDM) were measured using 4 transects (black lines), beginning at the edge of the open water (OW). Transects through areas of the wetland that did not terminate in wetland edge surrounded by cropland (dotted black line) were not measured. Percentage of the wetland perimeter surrounded by cropland (thick black wetland edge) was estimated in the field.

2.3.3 Other Wetland Variables Recorded

Additional wetland variables were recorded to account for the natural variation among study wetlands. Wetland surface area m^2 (area) was measured using satellite images and GPS assisted ground truthing. Wetland depth was recorded for each study wetland as being greater than or less than 1 m. Occurrences of cyanobacteria blooms were noted in study wetlands as a categorical presence or absence variable based on visual observation of wetlands.

2.3.4 Wetland Water Sampling

Water samples were collected from each study wetland at multiple time points throughout the summer, to be analyzed subsequently for pesticides, nutrients, and other water quality parameters. All water samples were collected by wading into the central water body of a wetland and filling containers dipped below the water surface, in front of any sediment that might be disturbed by carefully entering the wetland. Subsurface water samples were collected into two replicate amber glass bottles (2-L each) for pesticide analysis (see section 2.3.5). In addition, a 1-

L high-density polyethylene jug was filled with water for more general water quality analysis including that of nutrients (see section 2.3.6). Samples were stored in coolers in the field, later transferred to refrigerators at 4ºC, and extracted within 4 weeks of collection for pesticides and 48 hours for nutrients. Holding time limits as well as maintaining samples in dark and refrigerated conditions were followed to reduce pesticide degradation and metabolism as a result of UV light exposure or microbial growth (Acero et al., 2019; Bansal, 2012).

Water samples for pesticide analysis were collected from each wetland at three sampling periods throughout the growing season of 2018: late May, late June, and early July. The May sampling time was chosen to capture contamination from spring runoff containing pesticides from the previous year. By late May, wetlands had just thawed, and farmers were beginning to seed fields. The June sampling period was chosen to capture contamination from field applications in the current year. The July sampling period was chosen to represent later growing season conditions to assess pesticide concentrations before water levels were too low in study wetlands. Many PPR wetlands lose water throughout the summer, often becoming completely dry in the fall. Sample collection in early July ensured that the majority of wetlands still held water. Water samples collected for other water quality measurements were collected once from each wetland in late June, corresponding to the June sampling for pesticide analysis.

2.3.5 Pesticide Analysis of Water Samples

Water samples were analyzed for four neonicotinoid insecticides (imidacloprid, thiamethoxam, clothianidin, and acetamiprid) and six other systemic insecticides (chlorantraniliprole, cyantraniliprole, flonicamid, flubendiamide, flupyradifurone, and sulfoxaflor) at the National Hydrology Research Center, Environment and Climate Change Canada in Saskatoon, Saskatchewan. Analytical methods for LC/MS/MS were adapted from previously published methods (Xie et al., 2011). The same wetland water samples were also analyzed for 162 other pesticides at the Agriculture and Agri-Food Canada Trace Residue Analysis and Immunochemistry Laboratory, in Lethbridge, Alberta. Samples with pesticide concentrations (μg/L) below the limit of quantification (LOQ) were treated as non-detections (zeroes) and not assigned a value between zero and the LOQ in order to avoid overestimating total pesticide concentrations of the 172 pesticides analyzed. A list of the LOQs and recoveries of spiked samples for all pesticides analyzed can be found in Appendices A and B. Laboratory

blanks were all below detection and sample data were not recovery corrected. Total pesticide concentration of the 172 pesticides were calculated as the sum of all quantified pesticides in a wetland at a given sampling time.

2.3.6 Pesticide Toxicity Index (PTI) Calculation

The pesticides detected in wetlands belong to a number of pesticide classes and have a wide range of potential toxicities to aquatic life. To assess the relative toxicity of each pesticide mixture detected in study wetlands, Pesticide Toxicity Indices (PTIs) were calculated by summing the ratios of individual pesticide concentrations detected relative to that chemical's hazard concentration (HC_5) protective of 95% of the aquatic taxa drawn from an acute or chronic species sensitivity distribution (SSD) which was populated with published values from aquatic organism toxicity assays (Nowell et al., 2014). In cases where less than 7 values were available for use in SSDs, minimum toxicity values representing the most sensitive published toxicity value were used in place of an HC_5 (Appendix C). To assess the risk associated with pesticide contamination over the entire growing season, full growing season PTIs were calculated. For these full growing season PTIs, maximum concentrations of pesticides detected in May, June, and July were used to calculate acute PTIs and average concentrations detected across the 3 sampling periods were used to calculate chronic PTIs. To assess the risk associated with pesticide contamination that the June sampled aquatic invertebrate community was exposed to, mid-summer acute and chronic PTIs were calculated with maximum and mean May and June pesticide concentrations only. PTIs were only calculated when pesticide data was available for at least two of the sampling periods used for calculation. For this reason, missing pesticide data and insufficient water levels for pesticide sampling limited calculation of full growing season PTIs to 32 wetlands and mid-summer PTIs to 27 wetlands.

$$\textit{Average acute PTI} = \sum_{i=1}^{n} \frac{\textbf{maximum concentration in May,June,and July}}{\textbf{acute HC5}} \qquad \text{(Eqn. 2.2)}$$

$$\textit{Average chronic PTI} = \sum_{i=1}^{n} \frac{\textbf{mean concentration for May,June,and July}}{\textbf{chronic HC5}} \qquad \text{(Eqn. 2.3)}$$

$$\textit{Mid-summer acute PTI} = \sum_{i=1}^{n} \frac{\textbf{maximum concentration in May and June}}{\textbf{acute HC5}} \qquad \text{(Eqn. 2.4)}$$

$$\textit{Mid-summer chronic PTI} = \sum_{i=1}^{n} \frac{\textbf{mean concentration for May and June}}{\textbf{chronic HC5}} \qquad \text{(Eqn. 2.5)}$$

2.3.7 Nutrient and General Water Quality Analysis

Water samples collected in the June sampling period were also analyzed for nutrients and standard water quality parameters at the Water Security Agency, Government of Saskatchewan, Saskatoon, Saskatchewan including ammonia-nitrogen, bicarbonate, calcium, carbonate, dissolved chloride, conductivity, dissolved fluoride, iron, magnesium, manganese, non-purgeable organic carbon (NPOC), pH, phenol alkalinity, ortho phosphorus, total phosphorus (TP), potassium, sodium, dissolved sulfate, total alkalinity, total dissolved solids (TDS), total hardness, total nitrogen (TN), and total organic carbon (TOC).

2.3.8 Aquatic Invertebrate Collection

Aquatic invertebrate samples were collected at the same time as the June water sampling. At this time, wetlands were in a period of peak productivity and insect emergence. Samples were collected using the Canadian Aquatic Biomonitoring Network (CABIN) Protocol for wetlands (Environment and Climate Change Canada, 2018), with a 400-μm mesh D-frame sweep net. In an area of the wetland with emergent and submergent aquatic plants, the net was dipped beneath the water and moved in a zigzag pattern back and forth as the sampler walked forward, moving the net up and down through the water column and tapping the substrate to stir up benthic invertebrates. This motion was continued through the area of emergent and submergent aquatic plants for 2 minutes.

Aquatic invertebrate samples containing debris and vegetation were allowed to drain in the net before being transferred to 1-L plastic sample containers. Samples were preserved with ethanol in the field, adding 95% ethanol to sample containers to achieve approximately 70% ethanol in the preserved samples.

2.3.9 Aquatic Invertebrate Subsampling and Identification

Aquatic invertebrate samples were later processed, subsampled, and identified in the laboratory at the University of Saskatchewan using a modified version of the CABIN Laboratory Methods for Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples

(Environment and Climate Change Canada, 2014) with a Marchant box (Marchant, 1989) for aquatic invertebrate subsampling.

Samples were first rinsed by gently mixing each sample in a bucket of excess water. Large debris and vegetation were removed from the sample after thoroughly rinsing invertebrates and sediment on the debris/vegetation back into the sample being washed. Samples were then poured through a 400-μm mesh sieve, removing fine sediment from the sample.
Before beginning the process of subsampling, any large invertebrate ≥ 1.5 cm was removed and sorted to order to be identified later. This amendment to the original CABIN protocol was included due to the presence of large invertebrates such as Lymnaeidae and Belistomatidae that were not effectively subsampled using Marchant box cells. Additionally, by removing invertebrates ≥ 1.5 cm, large soft bodied invertebrates such as those from the order Odonata, were less likely to be damaged which would have led to difficulties during later identification.

Rinsed samples were transferred into a 100-cell Marchant box, filling the box with enough water to fill all Marchant box cells. After securing the lid on the Marchant box, the box was inverted, agitated, and quickly inverted again to best distribute invertebrates evenly. A random number generator was then used to select multiple cells from the 10 x 10 grid of 100 Marchant box cells for sorting and later identification. The contents of the first selected cell were then removed and transferred to a petri dish. Working under a dissecting microscope, all invertebrates from the selected cell were sorted into vials containing 70% ethanol by order and tallied. The original CABIN protocol does not include zooplankton, but was amended for the current study by sorting and recording abundance of three zooplankton taxa that were particularly abundant in our study wetlands (Cladocera, Copepoda, and Ostracoda) from the first randomly selected cell only.

After completion of the first cell, additional cells were randomly selected for sorting and tallying of all macroinvertebrates. Once a cell was started, it was always sorted in entirety. Randomly selected cells were sorted until two criteria were reached: 1) a minimum of 5 cells were sampled and 2) a minimum of 300 *insect* macroinvertebrates had been tallied. The original CABIN protocol requires a minimum of 300 macroinvertebrates, not necessarily insects. This modification was made to the protocol to ensure that sufficient diversity was captured in wetlands that were heavily dominated by Gastropoda and Amphipoda. In this modified protocol,

non-insect taxa were still sorted and tallied as were insect taxa, but only the insect taxa counted toward the 300 macroinvertebrate minimum.

After sorting of invertebrates to order, macroinvertebrates were identified to varying taxonomic levels using a dissecting microscope. Taxonomic keys (Clifford, 1991) as well as consultation with Iain Phillips, an aquatic invertebrate taxonomy specialist at the Water Security Agency, Government of Saskatchewan, Saskatoon, Saskatchewan were used for identification. Most insects were identified to genus, with the exception of Diptera and non-Corixidae Hemiptera which were only identified to family.

To calculate estimated abundance of each zooplankton and macroinvertebrate taxa in each sample based on the number of organisms in the subsample, the following equations were used:

$$\textit{Zooplankton taxa abundance} = \textit{number of organisms in cell}\ 1 \times 100\ \textit{cells} \qquad \text{(Eqn. 2.6)}$$

$$\textit{Macroinvertebrate taxa abundance} = (\textit{total number of organisms} \geq 1.5\ \textit{cm}) + \left(\frac{number\ of\ organisms < 1.5\ cm}{number\ of\ cells\ counted} \times 100\ \textit{cells}\right) \qquad \text{(Eqn. 2.7)}$$

2.3.10 Aquatic Invertebrate Community Indices

Occurrence and abundances of zooplankton and macroinvertebrates measured in wetlands were used to calculate aquatic invertebrate community composition indices. Richness (*S*) was determined for each wetland as the number of macroinvertebrate taxa identified in each wetland. Shannon's diversity (*H*) was calculated for each wetland in which p_i is the proportion of *S* made up of the *i*th macroinvertebrate taxa.

$$H = -\sum_{i=1}^{S} p_i \ln p_i \qquad \text{(Eqn. 2.8)}$$

Shannon's evenness (E_H) was calculated for each wetland by dividing *H* by H_{max} (here $H_{max} = \ln S$).

$$E_H = \frac{H}{H_{max}} = \frac{H}{\ln S} \qquad \text{(Eqn. 2.9)}$$

Berger-Parker dominance (*d*) was calculated for each wetland by dividing the number of individuals in the most abundant macroinvertebrate taxa (N_{max}) by the total number of individuals in all macroinvertebrate taxa (*N*).

$$d = \frac{N_{max}}{N} \qquad \text{(Eqn. 2.10)}$$

Hilsenhoff Biotic Index (*HBI*) was calculated for each wetland where x_i is the number of individuals in the *i*th macroinvertebrate taxon, t_i is the tolerance value of the *i*th macroinvertebrate taxon, and *n* is the total number of macroinvertebrates in the sample (Mandaville, 2002).

$$HBI = \frac{\sum x_i t_i}{n} \qquad \text{(Eqn. 2.11)}$$

The functional feeding group of each macroinvertebrate taxon identified was determined based on classification in the literature (Mandaville, 2002), allowing us to calculate the total abundance and relative abundance of macroinvertebrates in each functional feeding group defined as collector-gatherers, predators, scrapers, shredders, and omnivores. Relative abundance of each of these feeding groups was calculated by dividing the abundance of macroinvertebrates in a feeding group by the total abundance of macroinvertebrates in a sample.

In addition to the above biotic indices and functional feeding groups, total abundance and relative abundance of groups of invertebrate taxa were used as endpoints in analyses. These included zooplankton, Cladocera, Copepoda, Ostracoda, macroinvertebrates, insects, Diptera, Coleoptera, Odonata, Hemiptera, and Gastropoda. Relative abundances of zooplankton taxa were calculated by dividing the abundance of organisms in each zooplankton taxon by total zooplankton abundance. Relative abundances of insect and other macroinvertebrate taxa were calculated by dividing the abundance of organisms in each taxon by total macroinvertebrate abundance.

2.3.11 Statistical Analysis

2.3.11.1 Multivariate Relationships Between Wetland Disturbance and Water Quality

Multivariate and univariate statistical techniques performed in R Studio version 1.1.456 were used to analyze the relationship between natural wetland vegetation disturbance and water quality parameters measured in June water samples from 33 wetlands. Full growing season chronic PTI calculated for 32 wetlands was also considered to be a component of water quality and was included in the multivariate water quality analysis. Preliminary data exploration indicated that chronic PTI was more relevant and performed better in models. For these reasons, only chronic PTIs was used in the following analyses.

A number of water quality parameters measured in the 32 wetlands with full growing season chronic PTI were found to be collinear, with Pearson's r-correlation coefficients ≥ 0.70 (Appendix D). For multivariate analysis, covariates were removed, maintaining 11 water quality parameters (chloride, conductivity, fluoride, iron, manganese, NPOC, pH, total phosphorus, potassium, total nitrogen, and full growing season chronic PTI) with correlation coefficients < 0.07. Multivariate analyses were performed in R using the package vegan. A detrended correspondence analysis (DCA) performed on the 11 water quality parameter data indicated that linear methods were optimal for multivariate analysis (DCA1 = 0.26). A partial redundancy analysis (RDA) was performed on water quality parameters scaled to a mean of 0 and standard deviation of 1, with total zone width, vegetation disturbance, percent crop, wetland depth, crop type, and wetland area as constraining variables and block (grouping of neighboring fields) as a conditional variable. Pearson's r-correlation indices of constraining variables were all less than 0.70 (Appendix E) confirming non-collinearity of the variables. Significance of the RDA and individual constraining variables were assessed with permutation tests. The first two axis dimensions of the RDA were plotted to visualize relationships.

2.3.11.2 Linear Univariate Relationships Between Wetland Disturbance and Pesticide Contamination

To more closely examine the effect of wetland disturbance on pesticide contamination, univariate methods were employed, performing linear mixed effect models of pesticide concentration and PTI.

For analysis of pesticide concentrations in relation to wetland disturbance, the 34 study wetlands were sampled during three sampling periods throughout the growing season. However, due to some missing samples and wetlands with insufficient water levels in the late summer sampling period, not all 34 wetlands had pesticide data for all three sampling periods, resulting in 91 observations. Total pesticide concentrations (μg/L) measured in each water sample were cube root transformed to improve normality of the distribution. A global model assessing the response in total pesticide concentrations included the fixed effects of zone width, vegetation disturbance, percent crop, sampling period, wetland depth, crop type, and wetland area and the random effects of block and wetland ID (unique identifier given to each study wetland). Pearson's r-correlation coefficients of continuous fixed effects were all less than 0.70 (Appendix F) confirming non-collinearity of the variables.

For analysis of PTI, in relation to wetland disturbance, chronic PTIs calculated from pesticide concentrations in 32 of the 34 study wetlands were used in the following analysis. Chronic PTI was log-transformed to improve normality of the distribution. A global model assessing the response in chronic PTI included the fixed effects of zone width, vegetation disturbance, percent crop, wetland depth, crop type, and wetland area, as well as block as a random effect. Pearson's r-correlation coefficients of continuous fixed effects were all less than 0.70 (Appendix E) confirming non-collinearity of the variables.

Model selection was then performed on each global model guided by Akaike's Information Criterion corrected for small sample size (AICc) (Burnham & Anderson, 2002) (Appendix G & H) using the "dredge" function from the R package MuMIn (Barton´, 2020). The model with the lowest AICc score or those with $\Delta AICc < 2$ were considered as final models, using model averaging for all models with $\Delta AICc < 2$. Only final models or averages of final models found to have lower AICc values than null models were further analyzed for model results.

2.3.11.3 Effect of Vegetation Disturbance and Other Variables on the Aquatic Invertebrate Community

Multivariate and univariate techniques were used to analyze potential effects of natural wetland vegetation disturbance, pesticides, and other water quality parameters on aquatic invertebrate comminutes in wetlands. Although invertebrate samples were collected from 33 study wetlands, one or more water samples were missing from 6 wetlands, therefore, only the remaining 27 wetlands with full invertebrate, pesticide, and other water quality data were used for the following analysis.

Univariate techniques were used to assess multiple aquatic invertebrate community composition endpoints including well establish biotic indices (richness, Shannon's diversity, Shannon's evenness, Berger-Parker dominance, and Hilsenhoff Biotic Index) as well as total and relative abundance of multiple invertebrate groups (zooplankton, macroinvertebrates, insects, Cladocera, Copepoda, Ostracoda, Diptera, Coleoptera, Odonata, Hemiptera, and snails (Gastropoda), and the ratio of zooplankton to macroinvertebrates. Abundance of Amphipoda, Trichoptera, and Ephemeroptera were not included due to these taxa being relatively uncommon and poorly distributed across study wetlands (for example, only 24% of wetlands had Ephemeroptera present). Aquatic invertebrate endpoints that were not normally distributed were transformed for normal distribution. Global linear models were created for each aquatic invertebrate endpoint using zone width, vegetation disturbance, percent crop, wetland depth, crop type, wetland area, occurrence of cyanobacteria blooms, conductivity, pH, NPOC, total nitrogen, total phosphorus, log transformed chronic PTI, and block as fixed effects. Pearson's r-correlation coefficients of continuous fixed effects were all less than 0.70 (Appendix I). Preliminary data exploration suggested that block was not a strong predictor of aquatic invertebrate endpoints, but was included as a fixed effect, to avoid overfitting the models in a random effects structure. Relationships between acute PTI or chronic PTI and aquatic invertebrate endpoints were also explored, revealing that acute PTI was less predictive in models. For this reason, only chronic PTI was used to summarize pesticide mixture toxicity in the following analyses. Model selections were then performed on global models guided by AICc (Burnham & Anderson, 2002) (Appendix J) using methods identical to those described in section 2.3.11.2.

Multivariate techniques where then used to relate natural wetland vegetation disturbance, pesticide toxicity, and other water quality parameters to multivariate aquatic invertebrate data. Two separate analyses were performed: one of multivariate aquatic invertebrate taxon abundance data and the other of multivariate relative aquatic macroinvertebrate functional feeding group abundances. DCAs performed on both of these datasets indicated that linear methods were appropriate for multivariate analysis (DCA1 = 2.84 and DCA1 = 1.98). Two separate partial RDAs were performed; one on scaled Hellinger transformed aquatic invertebrate taxa abundance data, and the other on scaled Hellinger transformed relative aquatic macroinvertebrate functional feeding group abundances in the R package "vegan" (Oksanen et al., 2020). Each partial RDA contained the constraining variables of zone width, vegetation disturbance, percent crop, wetland depth, crop type, wetland area, occurrence of cyanobacteria blooms, conductivity, pH, NPOC, total nitrogen, total phosphorus, and chronic PTI, as well as block as a conditional variable. Pearson's r-correlation indices of continuous constraining variables were all less than 0.70 (Appendix I). Model selection using the "ordistep" function with pseudo-AIC (Bierwagen et al., 2018; Borcard et al., 2011; Calazans & Bocchiglieri, 2019; Kaestli et al., 2017) was then performed to obtain the most parsimonious partial RDA model with a reduced number of constraining variables for each of the two analyses. Significance of RDAs and individual constraining variables were assessed with permutation tests. The first two axis dimensions of RDAs were plotted to visualize relationships.

2.4 Results

2.4.1 Effect of Wetland Vegetation Disturbance on Water Quality

The degree of wetland vegetation disturbance of 34 PPR wetlands was assessed by measuring vegetation zone width and vegetation disturbance. Zone width ranged between 1.8 m and 40.9 m and vegetation disturbance, a measure of the loss or removal of natural vegetation cover, ranged between 24% and 92%. Vegetation disturbance and zone width were found to be significantly negatively associated with one another ($p < 0.001$) in a linear model, however not strongly correlated based on Pearson's correlation coefficient (0.52) (Figure 2.3).

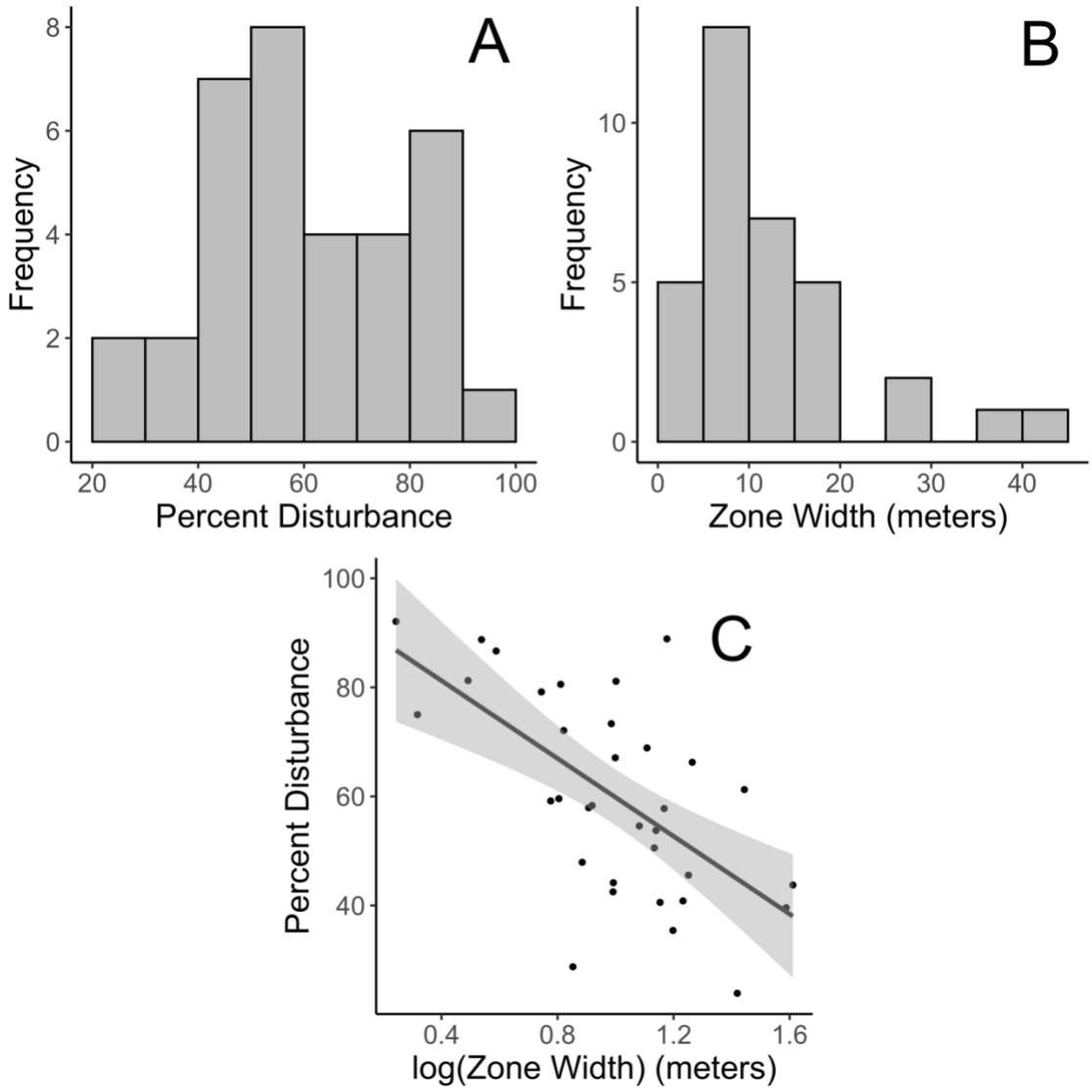

Figure 2.3. Distribution of wetland vegetation zone widths (A) and percent disturbance (vegetation disturbance) (B) and their relationship with each other (C) for 34 study wetlands in the PPR of Saskatchewan. Percent vegetation disturbance and vegetation zone width were significantly negatively associated with one another ($p < 0.001$) in a linear model, however not strongly correlated based on Pearson's correlation coefficient (0.52).

Water samples from the 34 study wetlands were analyzed for concentrations of 172 current use pesticides, detecting 24 different pesticides including 4 neonicotinoid insecticides, a pyrethroid insecticide, a pyridine insecticide, 2 ryanoid class insecticides, 10 herbicides, and 6 fungicides. Quantifiable concentrations of pesticides were measured in all 34 wetlands. Most wetlands contained mixtures of pesticides with as many as 9 pesticides detected in a single wetland in June. On average, individual wetlands contained 3, 4, and 3 pesticides in May, June,

and July, with neonicotinoid insecticides being the most frequently detected pesticide group, followed by the herbicides. Neonicotinoids and herbicides were detected in 74.2% and 71.4% of samples, respectively. The neonicotinoid clothianidin was the most frequently detected pesticide, found in 59.1% of samples and at a maximum concentration of 0.039 µg/L. The most frequently detected herbicide was 2,4-D, found in 49.5% of samples and at a maximum concentration of 1.541 µg/L (Table 2.1).

Table 2.1. Geometric mean concentrations of pesticides (µg/L) quantified in water sampled from 34 study wetlands in the PPR of Saskatchewan in May, June, and July of 2018. Concentrations below LOQs (Appendix A) were set to 0.0001 µg/L for calculation of geometric means in this table.

Pesticide Class	Pesticide	Geometric Mean (µg/L)	Max Concentration (µg/L)	Percent Detection
Neonicotinoid Insecticides	Imidacloprid	< 0.001	0.041	25.3
	Thiamethoxam	< 0.001	0.027	20.9
	Clothianidin	0.001	0.039	58.2
	Acetamiprid	< 0.001	0.003	3.3
	All Neonicotinoids	**0.003**	**0.056**	**74.7**
Other Insecticides	Chlorantraniliprole	< 0.001	0.003	6.6
	Cyantraniliprole	< 0.001	0.001	1.1
	Flonicamid	< 0.001	0.002	4.4
	Bifenthrin	< 0.001	0.085	2.2
	All Other Insecticides	**< 0.001**	**0.085**	**14.3**
Herbicides	2,4-Dichlorophenoxyacetic acid	0.003	1.541	49.5
	MCPA (2-methyl-4-chlorophenoxyacetic acid)	< 0.001	2.886	13.2
	Bromoxynil	< 0.001	0.293	4.4
	Clopyralid	0.001	1.248	37.4
	Dicamba	< 0.001	0.034	2.2
	Fluroxypyr	0.001	1.547	37.4
	Imazamethabenz	< 0.001	0.559	1.1
	Imazethapyr	< 0.001	0.119	1.1
	Quinclorac	< 0.001	0.083	4.4
	Triallate	< 0.001	0.076	2.2
	All Herbicides	**0.018**	**6.009**	**71.4**
Fungicides	Iprodione	< 0.001	0.037	1.1
	Metalaxyl	< 0.001	0.041	4.4
	Picoxystrobin	< 0.001	0.033	2.2
	Prothioconazole-Desthio	< 0.001	0.089	12.1
	Tebuconazole	< 0.001	0.093	2.2
	Trifloxystrobin	< 0.001	0.477	16.5
	All Fungicides	**< 0.001**	**0.477**	**30.8**

To assess the toxicity of these pesticide mixtures and the effect they had on water quality, full growing season PTIs were calculated for 32 of the study wetlands (Table 2.2 & Figure 2.4). Acute full growing season PTIs ranged from 0.001 to 4.913 with two PTIs exceeding the threshold of 1.0 (6% of wetlands). Chronic full growing season PTIs ranged from 0.132 to 68.486 with 28 PTIs exceeding the threshold of 1.0 (88% of wetlands) (Figure 2.4). Insecticide concentrations contributed the most to both acute and chronic PTIs, on average making up 82.3% of acute PTIs and 94.4% of chronic PTIs. Fungicides on average contributed to 16.4% and 5.0% of acute and chronic PTIs, respectively, while herbicides on average contributed to 1.2% and 0.7% of acute and chronic PTIs, respectively (Figure 2.4). The mean concentrations of all other water quality parameters measured in the 32 study wetlands are provided in Table 2.2.

Table 2.2. Summary of wetland water quality variables measured in 32 wetlands in the PPR of Saskatchewan in 2018. Water quality variables in **bold** are those included in a partial RDA (Figure 2.5) after removal of covariates (Pearson's correlation coefficient > 0.07) (Appendix D).

Water Quality Parameter	**Mean ± SD**
Ammonia-N (mg/L)	0.40 ± 0.73
Bicarbonate (mg/L)	370.56 ± 142.85
Calcium (mg/L)	91.09 ± 27.94
Carbonate (mg/L)	43.09 ± 34.75
Chloride (mg/L)	23.93 ± 15.22
Conductivity (µS/cm)	1360.16 ± 538.01
Fluoride(mg/L)	0.19 ± 0.05
Iron (mg/L)	0.62 ± 1.08
Magnesium (mg/L)	132.38 ± 85.02
Manganese (mg/L)	0.37 ± 0.67
NPOC (mg/L)	37.70 ± 8.42
pH	8.84 ± 0.52
Phenol Alkalinity (mg/L $CaCo_3$)	35.96 ± 28.94
Ortho Phosphorus (mg/L)	0.85 ± 0.50
Total Phosphorus (mg/L)	1.10 ± 0.64
Potassium (mg/L)	38.50 ± 11.07
Sodium (mg/L)	44.28 ± 26.59
Sulfate (mg/L)	426.30 ± 367.81
Total Alkalinity (mg/L $CaCo_3$)	375.66 ± 95.86
TDS (mg/L)	1170.34 ± 517.25
Total Hardness (mg/L $CaCo_3$)	772.63 ± 393.81
Total Nitrogen (mg/L)	4.02 ± 1.42
TOC (mg/L)	40.09 ± 10.32
Full Growing Season Acute PTI	0.29 ± 0.93
Full Growing Season Chronic PTI	6.29 ± 12.38

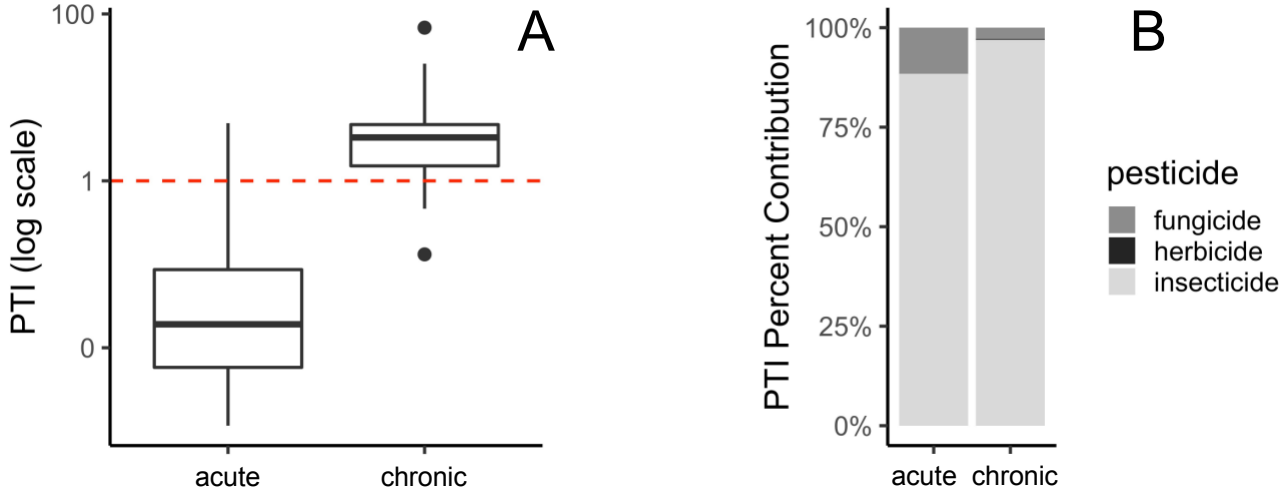

Figure 2.4. Acute and chronic full growing season PTI ranges and percent contribution of different pesticide classes. A) Boxplot of acute and chronic full growing season PTIs. Horizontal red dashed line marks a PTI of 1, the risk threshold at which the pesticide mixture has met the HC_5. PTIs greater than 1 (red line) indicate increasing risk. B) Percent contribution of fungicides, herbicides, and insecticides to the total acute and chronic full growing season PTIs.

A partial RDA of water quality parameters with environmental and vegetation disturbance constraining variables measured in the 32 study wetlands (Figure 2.5) was significant ($F = 1.66$, $p = 0.012$), with total zone width found to be the only explanatory variable significantly influencing water quality ($F = 4.83$, $p = 0.001$) (Table 2.3).

Relationships between wetland disturbance and pesticide contamination were then further investigated in linear mixed effect models of pesticide concentrations and full growing season chronic PTI. In AICc based model selections, the null model of both pesticide concentration and chronic PTI were found to be among the best approximating models (Appendix G & H), therefore wetland disturbance variables were not found to be significant in their effect on pesticide contamination in wetlands.

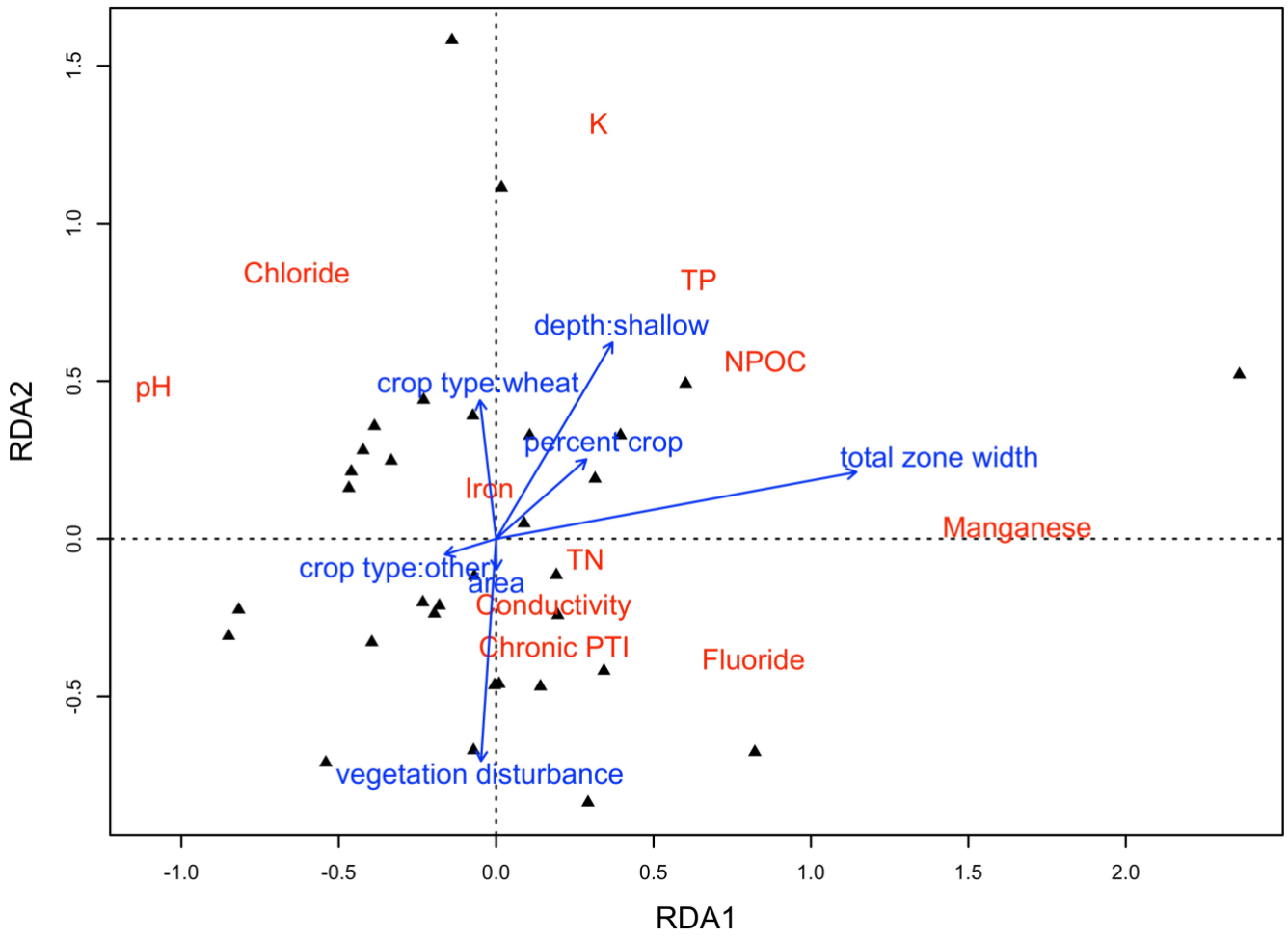

Figure 2.5. Partial RDA of scaled water quality variables in red (conductivity, fluoride, iron, manganese, non-purgeable organic carbon (NPOC), pH, total phosphorus (TP), potassium (K), total nitrogen (TN), and full growing season chronic PTI (Chronic PTI)) and 32 wetland sites (black triangles), constrained by environmental and disturbance variables in blue. This partial RDA was significant ($F = 1.66$, $p = 0.012$) as determined in a permutation test. Axis RDA1, capturing 17.6% of variance, was significant ($F = 5.38$, $p = 0.036$), however RDA2, capturing 6.9% of variance, was not significant ($p > 0.05$) as determined in permutation tests. Zone width was the only constraining variable that was significant in its influence on water quality ($F = 4.83$, $p = 0.001$) in a permutation test of RDA constraining variables (Table 2.3).

Table 2.3. Significant associations of environmental and vegetation disturbance variables with water quality of 32 Saskatchewan wetlands analyzed in a partial RDA (F = 1.66, p = 0.012) (Figure 2.5). Bolded values indicate statistical significance (p ≤ 0.05 *, p ≤ 0.01 **, p ≤ 0.001 ***) of the vectors determined through permutation tests.

Variables influencing water quality	[a]VIF	F	p-value	
Depth	2.54	1.508	0.159	
Crop Type	2.17	1.359	0.183	
Area	2.26	0.641	0.709	
Vegetation Disturbance	2.80	0.698	0.684	
Total Zone Width	2.25	4.832	**0.001**	***
Percent Crop	1.46	1.196	0.301	

[a]Variance Inflation Factor

2.4.3 Wetland Vegetation Disturbance, Water Quality, and Other Factors Influencing the Aquatic Invertebrate Community

Aquatic invertebrate communities of 27 study wetlands were sampled and processed, yielding 82 taxonomic groups of invertebrates identified to varying taxonomic levels. While at least 36 macroinvertebrate families were represented among these identified taxa, only 3 macroinvertebrate families were found to be common to all sampled wetlands (Chironomidae, Dytiscidae, and Corixidae). These three families were among the most abundant across all wetlands as measured by those with the highest median count (Figure 2.6) (Appendix K). Aquatic invertebrate abundances were used to calculate multiple biotic indices and community endpoints which are summarized in Table 2.4.

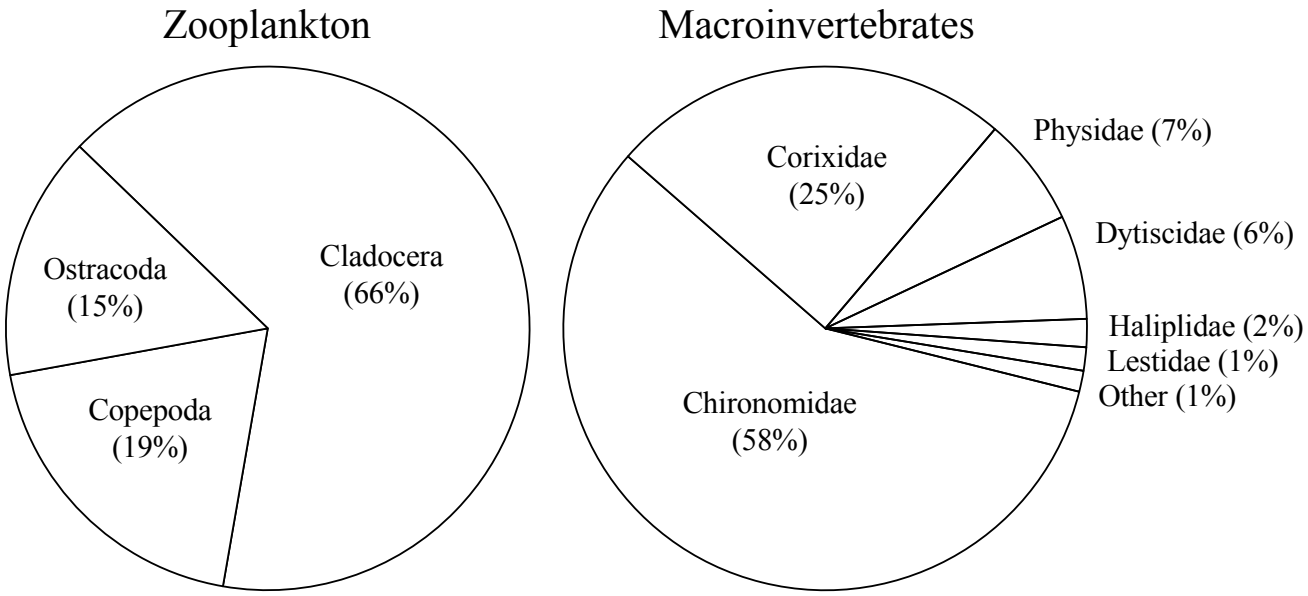

Figure 2.6. Percent median abundance of zooplankton taxa and the macroinvertebrate families common to more than half of 27 Saskatchewan wetlands sampled in June 2018 (Appendix K). The "Other" families that make up 1% of median macroinvertebrate abundance are Planorbidae, Hyalellidae, Lymnaeidae, Notonectidae, and Hydrachnidia which were not identified to family.

Table 2.4. Summary of median (range) and mean aquatic invertebrate community biotic indices including macroinvertebrate and zooplankton abundance, as well and richness, Shannon's diversity, Shannon's evenness, Berger-Parker dominance, and Hilsenhoff Biotic Index for macroinvertebrates collected from 27 Saskatchewan wetlands in June of 2018.

Aquatic Invertebrate Community Biotic Index or Endpoint	**Median (Range)**	**Mean ± SD**
Total Macroinvertebrate Abundance	5,704 (801 – 72,717)	9,232 ± 14,191
Total Zooplankton Abundance	31,666 (5,510 – 576,500)	64,935 ± 110,476
Richness	18 (8 - 28)	18.0 ± 5.5
Hilsenhoff Biotic Index	6.52 (5.47 - 7.86)	6.49 ± 0.65
Shannon's Diversity	1.47 (0.29 - 2.10)	1.29 ± 0.53
Shannon's Evenness	0.50 (0.12 - 0.67)	0.44 ± 0.16
Berger-Parker Dominance	0.55 (0.26 - 0.94)	0.59 ± 0.21

2.4.3.1 Effects on Aquatic Invertebrate Community Endpoints in Univariate Models

Linear models of aquatic invertebrate community endpoints were performed to assess their relationships with environmental, vegetation disturbance, pesticide toxicity, and other water quality variables (Appendix J). Mid-summer chronic PTI, which ranged from 0.20 to 100.73 and exceeded the risk threshold of 1.0 in 24 wetlands (89%) (Appendix L), was used as the pesticide toxicity variable in these models. AICc based model selection and model averaging resulted in 23 final models of aquatic invertebrate community endpoints in which the most parsimonious final models had lower AICc values than null models (Appendix J). The results of these 23 aquatic invertebrate community endpoint final models (Appendix M) are described below and summarized in a heat map which depicts the significance of each predictor variable in each final model (Figure 2.7).

Multiple vegetation disturbance variables were retained in aquatic invertebrate community endpoint models and found to be significant (Figure 2.7). Zone width was retained in 10 of the 23 final models and associated with significant increases in total and relative snail abundance ($p = 0.006$ and $p < 0.001$) as well as increased richness ($p = 0.031$). Vegetation disturbance, retained in 9 of the 23 final models was significantly associated with decreases in total and relative Odonata abundance ($p = 0.001$ and $p = 0.001$) and Hilsenhoff Biotic Index ($p = 0.020$) as well as an increase in total snail abundance ($p = 0.015$). Percent crop and crop type were each retained in 7 final models, results of which can be found in Appendix M.

Multiple water quality variables were found to have significant impacts on different aspects of aquatic invertebrate communities (Figure 2.7). The occurrence of cyanobacteria blooms was the most important predictor variable overall, retained in 18 of the 23 final models, more than any other predictor variable. Cyanobacteria blooms were associated with significantly lower Shannon's diversity ($p = 0.001$), Shannon's evenness ($p = 0.002$), total abundance of insects ($p = 0.001$), snails ($p = 0.001$), and Diptera ($p < 0.001$), and relative abundance of insects ($p < 0.001$), Diptera ($p < 0.001$), Hemiptera ($p = 0.002$), Copepods ($p = 0.010$), and Ostracods ($p < 0.001$). Cyanobacteria blooms were also associated with significantly greater Hilsenhoff Biotic Index ($p = 0.004$), Berger Parker Dominance ($p = 0.004$), and macroinvertebrate abundance ($p = 0.010$) (Appendix M).

Total nitrogen and pH were the second most retained water quality variables, each retained in 13 of the 23 models (Figure 2.7). Notably, increases in total nitrogen were

significantly associated with decreases in richness ($p = 0.001$), Shannon's diversity ($p = 0.016$), and Shannon's evenness ($p = 0.001$), and increased Berger-Parker Dominance ($p = 0.001$). Full model results for total nitrogen, pH, and other water quality variables retained in models including conductivity and NPOC can be found in Appendix M.

Estimated toxicity of the pesticide mixtures detected in wetlands quantified as mid-summer chronic PTI was significantly associated with changes in multiple aquatic invertebrate community endpoints. Mid-summer chronic PTI was retained in 11 of the 23 models and associated with significant declines in total abundance of insects ($p = 0.016$), relative abundance of insects ($p < 0.001$), Diptera ($p = 0.008$), and Copepods ($p = 0.013$), as well as a significant increase in relative snail abundance ($p = 0.005$) (Figure 2.7 – 2.9) (Appendix M).

Two environmental variables (wetland depth and wetland area) were included in global models and retained in 14 and 6 final models respectively (Figure 2.7). Model results for significant associations of these variables can be found in Appendix M. The wetland regional grouping of block was retained in 2 of the 23 models but not included in Figure 2.7 (Appendix M).

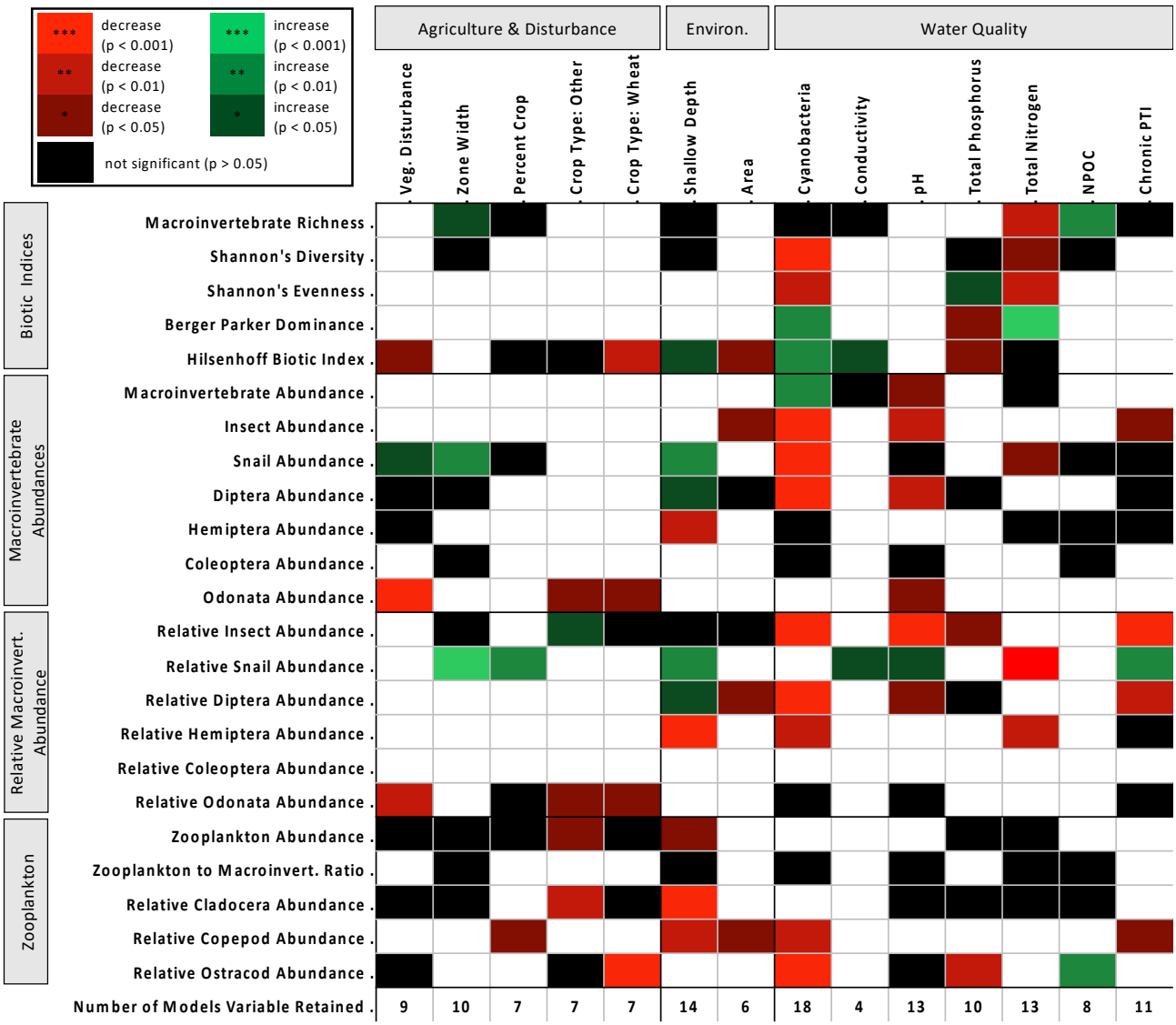

Figure 2.7. Heat map of predictor variables and their relative association with aquatic invertebrate community endpoints in 27 Saskatchewan wetlands assessed in 2018, as determined by separate linear models (Appendix J). Global models included 14 environmental, disturbance, pesticide, and other water quality predictor variables (zone width, vegetation disturbance, percent crop, crop type, wetland area, wetland depth, cyanobacteria bloom, conductivity, pH, total phosphorus, total nitrogen, NPOC, log chronic mid-summer pesticide toxicity index (Chronic PTI), and block. Filled in tiles represent variables retained in final best supported models using AICc guided model selection and model averaging. Shades of red indicate a significant negative relationship while shades of green indicate a significant positive relationship. Black indicates a variable retained in a final model that was not significant. The shade of the color indicates the degree of significance based on p values extracted from each parameter estimate (see legend in upper left of figure) (Appendix M).

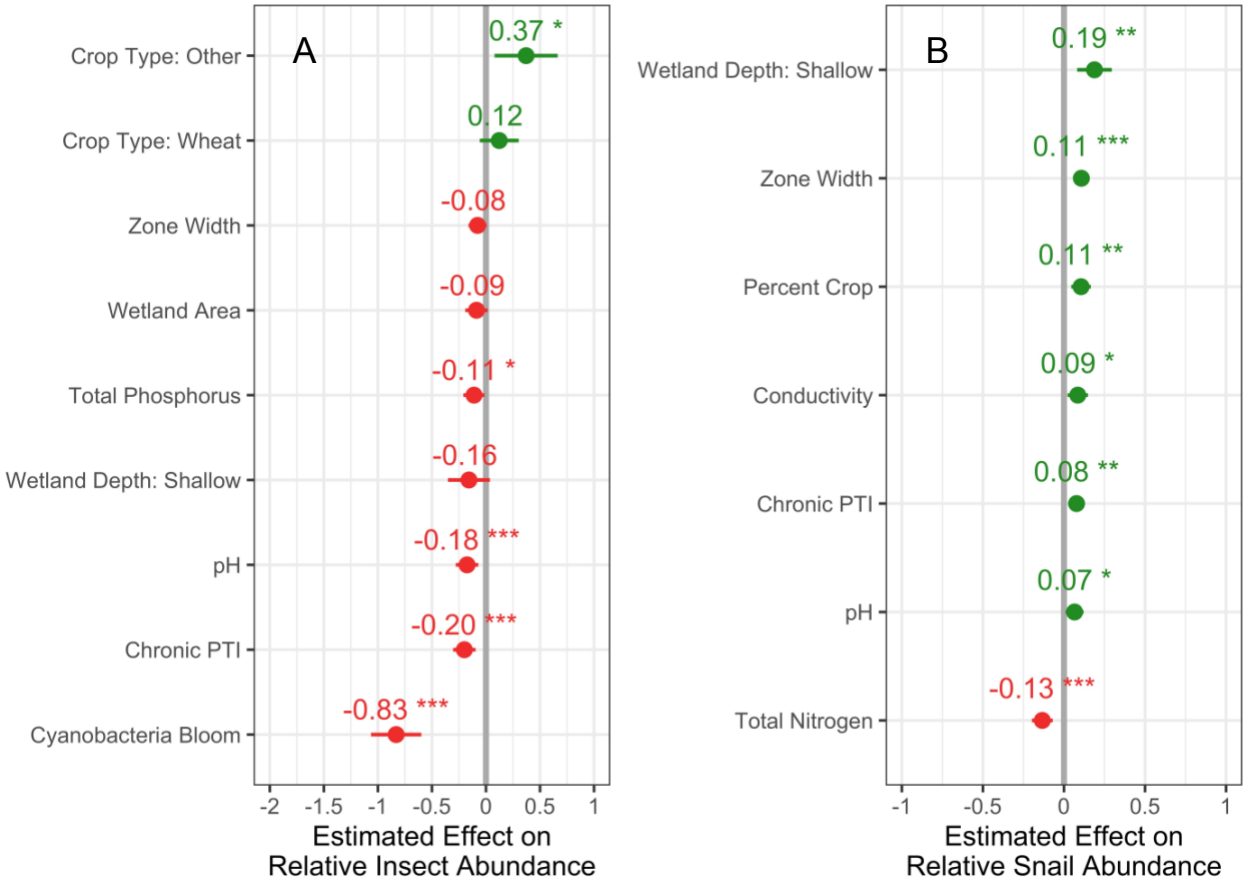

Figure 2.8. Estimated effect of disturbance, pesticide, and other water quality variables on relative insect (A) and relative snail (B) abundance determined through linear models of each aquatic invertebrate community endpoint followed by AICc based model selection (Appendix M).

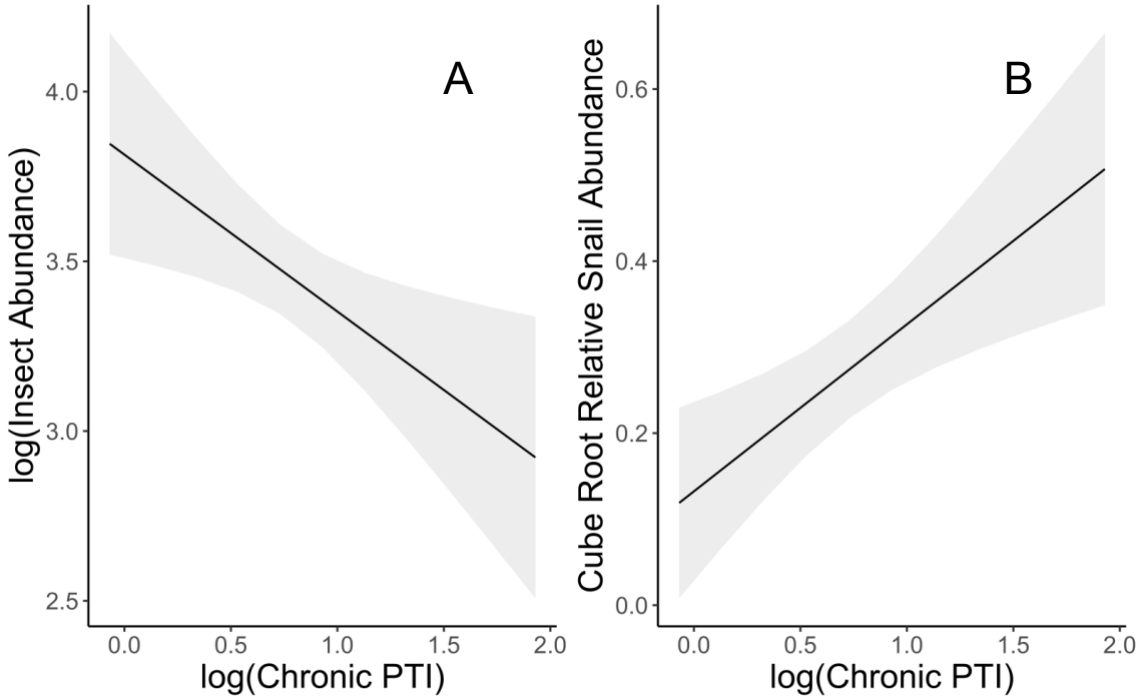

Figure 2.9. Linear models showing predicted effect of mid summer chronic PTI (log scale) on total insect abundance (A) and relative snail abundance (B) sampled from 27 Saskatchewan study wetlands in 2018. Mid-summer chronic PTI was negatively associated with total insect abundance ($p = 0.016$) in a linear model containing the other predictor variables area, cyanobacteria bloom, and pH. Mid-summer chronic PTI was positively associated with relative snail abundance ($p = 0.005$) in a linear model containing the other predictor variables zone width, percent crop, wetland depth, conductivity, pH, and total nitrogen (Appendix M).

2.4.3.2 Multivariate Analysis of Factors Influencing the Aquatic Invertebrate Community

Multivariate techniques were used to analyze taxa abundances at the lowest taxonomic units identified. A partial RDA was performed on these taxa abundances constrained by the same environmental, disturbance, pesticide, and other water quality variables as used in univariate models. After partial RDA model selection, four constraining variables were retained (pH, conductivity, total phosphorus, and NPOC) in a partial RDA that captured 29.6% of data variance in the first two axes. This partial RDA was found to be significant in a permutation test ($F = 2.70$, $p = 0.001$), as well as both of the first RDA axes (RDA1: $F = 5.35$, $p = 0.001$ and RDA2: $F = 2.89$, $p = 0.005$). Conductivity ($F = 2.48$, $p = 0.039$), total phosphorus ($F = 1.71$, $p =$

0.049), NPOC (F = 4.42, p = 0.002), and pH (F = 2.21, p = 0.020) were significantly associated with changes in aquatic invertebrate taxa abundances in this partial RDA, determined by a permutation test (Table 2.5) (Appendix N).

A partial RDA was also performed on relative functional feeding group abundance of macroinvertebrates constrained by the same environmental, disturbance, pesticide, and other water quality, variables as used in univariate models. After partial RDA model selection, five constraining variables were retained (wetland depth, cyanobacteria bloom, mid-summer chronic PTI, pH, and total nitrogen) in a partial RDA that captured 61.9% of data variance in the first two axes. This partial RDA was found to be significant in a permutation test (F = 6.66, p = 0.001), as well as the first RDA axis, RDA1 (F = 25.71, p = 0.001). Wetland depth (F = 5.20, p = 0.014), cyanobacteria blooms (F = 17.57, p = 0.001), and mid-summer chronic PTI (F = 5.06, p = 0.007) were significantly associated with changes in aquatic invertebrate feeding group abundances in this partial RDA, determined by a permutation test (Table 2.6 & Figure 2.10).

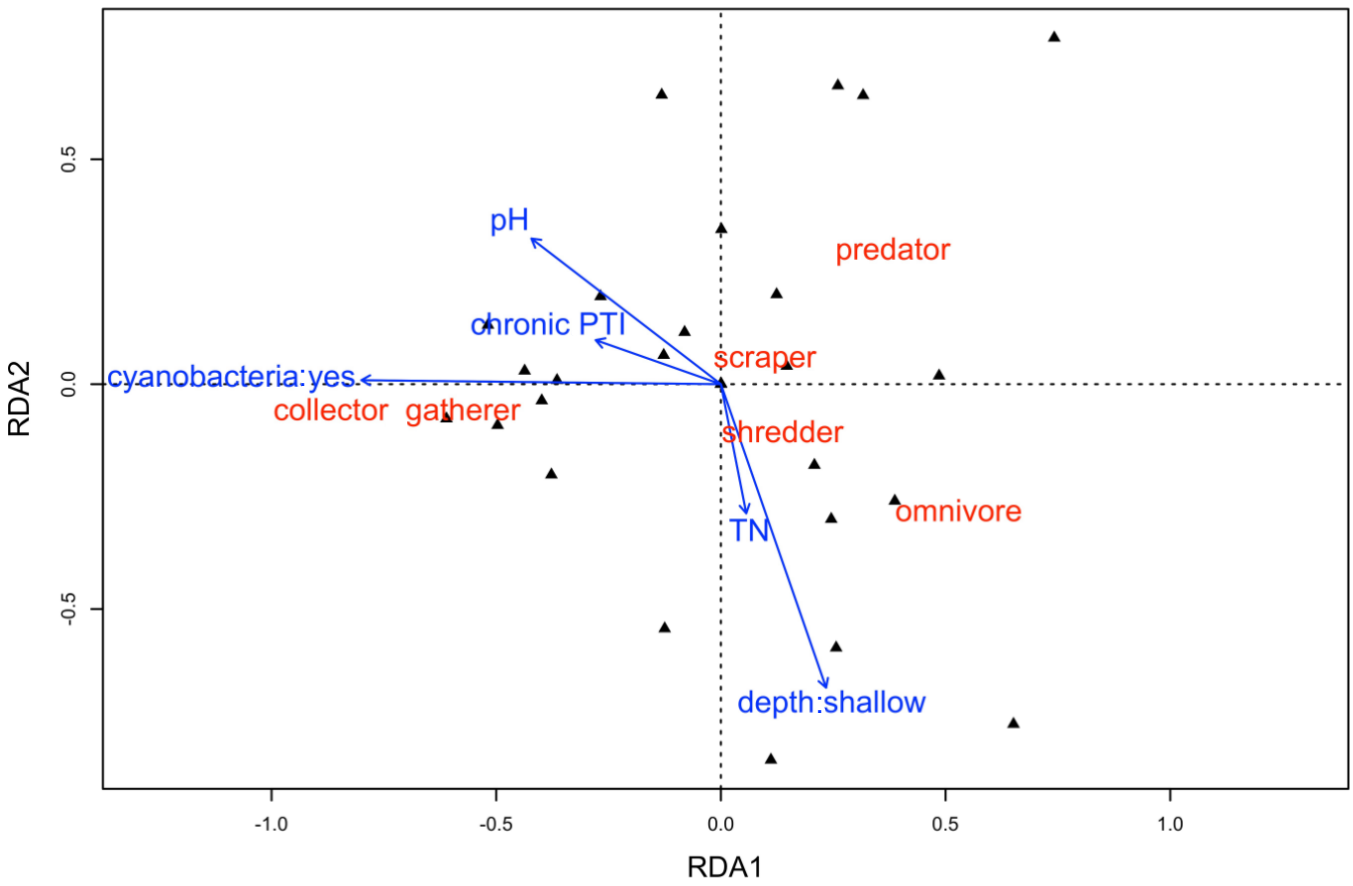

Figure 2.10. Partial RDA of scaled aquatic macroinvertebrate feeding group abundances (red) and 27 wetland sites (black triangles), constrained by total nitrogen (TN), pH, occurrence of cyanobacteria blooms (cyanobacteria: yes), shallow wetland depth (depth: shallow), and mid-summer chronic PTI (chronic PTI) (blue arrows). This partial RDA significantly explained 61.9% of variance ($F = 6.66$, $p = 0.001$), and the first RDA axis was significant ($F = 25.71$, $p = 0.001$). Wetland depth ($F = 5.20$, $p = 0.014$), cyanobacteria blooms ($F = 17.57$, $p = 0.001$), and mid-summer chronic PTI ($F = 5.06$, $p = 0.007$) were significantly associated with changes in aquatic invertebrate feeding group abundances (Table 2.6).

Table 2.5. Significant associations of water quality variables with taxa abundances of aquatic invertebrates sampled in 27 Saskatchewan wetlands in 2018, analyzed in a partial RDA ($F = 2.70$, $p = 0.001$) (Appendix N). Bolded values indicate statistical significance ($p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***) of the vectors determined through permutation tests.

Variables associated with aquatic invertebrate taxa abundances	[a]VIF	F	p-value	
Conductivity	3.19	2.48	**0.039**	*
NPOC	2.12	4.42	**0.002**	**
Total Phosphorus	2.25	1.71	**0.049**	*
pH	1.49	2.21	**0.02**	*

[a]Variance Inflation Factor

Table 2.6. Significant associations of environmental, pesticide, and other water quality variables on relative abundances of aquatic invertebrates functional feeding groups sampled in 27 Saskatchewan wetlands in 2018, analyzed in a partial RDA (F = 6.66, p = 0.001) (Figure 2.10). Bolded values indicate statistical significance (p ≤ 0.05 *, p ≤ 0.01 **, p ≤ 0.001 ***) of the vectors determined through permutation tests.

Variables associated with aquatic invertebrate functional feeding group abundances	[a]VIF	F	p-value	
Wetland Depth	1.83	5.20	0.014	*
Cyanobacteria	1.55	17.57	0.001	***
Chronic PTI	1.5	5.06	0.007	**
pH	1.38	2.67	0.069	
Total Nitrogen	2.60	2.79	0.053	

[a]Variance Inflation Factor

2.5 Discussion

Agriculture throughout the PPR of Canada has impacted wetlands both physically and chemically through disturbance of wetland vegetation and contamination with pesticides. This study examines how agricultural disturbances impact wetland health, assessed through changes in water quality and the aquatic invertebrate community. I hypothesized that disturbance of wetland vegetation would be associated with declines in water quality and that vegetation disturbance and changes in water quality due to agriculture would have significant effects on the aquatic invertebrate community of wetlands.

2.5.1 Effect of Wetland Vegetation Disturbance on Water Quality

Wetland vegetation disturbance, measured as zone width and loss of vegetation cover (vegetation disturbance), were found to be widespread in study wetlands. However, only zone width was significantly associated with wetland water quality parameters in a partial RDA. While zone width measured in this study can be reflective of the vegetation disturbance caused by agricultural activities around wetlands, it is also a product of other environmental factors and is therefore not a direct measurement of disturbance. PPR wetlands naturally range in zone width depending on wetland basin topography and basin fill (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). Wetlands with deeper basins and

steeper slopes may have compressed vegetation zones with shorter widths, while wetlands with shallower basins and more gradual slopes may have expanded vegetation zones and greater zones widths. As a result, the zone widths measured in this study are a product of the original zone widths of a wetland *and* the agricultural activities that have led to the removal of portions of these zones. Therefore, the changes in water quality found to be associated with zone width could be a product of environmental variation and not a direct effect of agriculture.

Pesticide contamination was widespread in study wetlands; however concentrations of neonicotinoids quantified in wetlands were generally lower than what has been found by a previous study of PPR wetlands (Main et al., 2014). Wetland sampling conducted by Main et al., 2014 was carried out during a period of wet climatic conditions compared with the sampling period of the current study which was relatively dry. Drier conditions and low levels of precipitation could explain relatively lower concentrations of neonicotinoids detected in surface waters (Anderson et al., 2013).

Pesticide concentrations quantified in wetlands were further investigated for their relationship with wetland vegetation disturbance in univariate models. Vegetation disturbance and zone width were not significantly associated with differences in concentrations of pesticides in wetlands or PTIs. Previous work has demonstrated that certain natural wetland vegetation composition in PPR wetland vegetation zones is associated with reduced neonicotinoid contamination in wetlands (Main et al., 2015). It is possible that vegetation disturbance and zone width, the metrics used in the current study, are less predictive of pesticide contamination than vegetation composition.

Another possible explanation for the lack of relationship between vegetation disturbance and pesticide contamination in this study is that vegetation zones of a wetland might not be effective buffers when degraded and not fully intact. Wetland vegetation zones are themselves part of the actual wetland. More permanent wetlands with central open water bodies, such as those selected for this study, naturally contain three distinct vegetation zones; the wet meadow, shallow marsh, and emergent deep marsh (Huel, 2000). Although the degree of disturbance measured in the vegetation zones of wetlands in this study varied, all 34 wetlands were found to have vegetation disturbance greater than 0. In other words, no study wetland had fully intact vegetation zones. Given that no significant relationship was found between vegetation disturbance and the widespread pesticide contamination detected in wetlands, it could be that vegetation zones that

are not fully intact are not sufficient in protecting wetlands from pesticide contamination. Instead, pesticide contamination might be better mitigated by vegetation buffers maintained or implemented beyond the vegetation zones of a wetland, in the upland area between a wetland and cropland.

2.5.2 Effects of Wetland Vegetation Disturbance, Water Quality, and other Factors on Aquatic Invertebrate Communities

Aquatic invertebrates have been used in biomonitoring of streams for many years and have been demonstrated to be responsive to a variety of stressors. We hypothesized that disturbance of wetland vegetation as well as water quality changes, including pesticide contamination, would be associated with significant changes in aquatic invertebrate communities of study wetlands.

In this study, greater zone width, a possible sign of a less disturbed wetland, was associated with greater macroinvertebrate richness. Greater richness of aquatic invertebrates has been associated with ecosystem stability and the absence of disturbance in streams (Compin & Céréghino, 2003; Death, 2002; Death & Winterbourn, 1995; Roy et al., 2003). While fewer studies have examined the effects of disturbance on the richness of wetland aquatic invertebrate communities, one study found that wetlands surrounded by cattle grazing had greater aquatic invertebrate richness compared to wetlands surrounded by disking, a practice often used in conjunction with soil tillage (Davis & Bidwell, 2008). Although the effects of cattle grazing were not evaluated in the present study, soil disturbance in the form of tillage or disking is a practice used in annual crop production such as the canola and wheat production that took place on fields in this study. It is possible that the annual crop production examined in this study had similar negative effects on aquatic invertebrate community richness as observed by Davis & Bidwell (2008). The findings here suggest that wetlands with greater zone width might support more stable aquatic environments that are not as impacted by the farming practices surrounding them, resulting in greater macroinvertebrate richness.

Loss of vegetation cover in wetland vegetation zones (vegetation disturbance) was significantly associated with declines in total and relative Odonata abundance. A previous study investigating the impacts of grazing disturbance around Canadian PPR wetlands found that removal of emergent vegetation by cattle grazing was associated with a decrease in abundance of Odonata (Foote & Hornung, 2005). The authors concluded that the presence of emergent wetland

plants was crucial for Odonata breeding, and that removal or grazing of these plants negatively affected Odonata abundance and diversity due to loss of breeding habitat. Several Odonata species found in Saskatchewan PPR wetlands mate while resting on emergent vegetation and oviposit eggs directly into the stalks of these plants, making wetland vegetation an integral part of the Odonata lifecycle (Sawchyn, 1971). Odonata are predatory insects in both their aquatic and adult life stages and serve as beneficial insects, consuming mosquitos (May, 2019; Urabe et al., 1990) and crop pests (Ghahari et al., 2009). Protection of existing PPR wetland vegetation could therefore enhance the ecosystem services that wetlands provide to humans and agriculture.

Multiple water quality parameters were strongly associated with a number of wetland aquatic invertebrate community endpoints in this study. The occurrence of cyanobacteria blooms was found to be the strongest predictor variable in multiple models of aquatic invertebrate endpoints, associated with declines in Shannon's diversity and abundance of a number of invertebrate taxa as well as an increase in Berger-Parker dominance. Cyanobacteria blooms were also associated with a shift in relative functional feeding group composition in a partial RDA. Overall, wetlands with cyanobacteria blooms were less diverse, less stable, and contained lower abundances of many aquatic invertebrates compared with wetlands without cyanobacteria blooms.

Some cyanobacteria blooms or Harmful Algal Blooms (HABs) are potentially toxic to aquatic life due to cyanotoxin production and creation of anoxic conditions (Camargo & Alonso, 2006; Hudnell et al., 2008). While the occurrence of HABs has largely been attributed to abiotic factors such as loading of aquatic environments with excess nutrients, more recent work has linked HABs to biotic factors, which, in conjunction with abiotic factors, increase the likelihood of a HAB (Nelson et al., 2018; Wilk-Woźniak, 2019). There is evidence that aquatic systems with more stable communities are less prone to HABs (Kim et al., 2021). Consequently, it is unclear whether the cyanobacteria associated declines in diversity and increases in dominance observed in this study are effects of the cyanobacteria blooms or actually predictors of the cyanobacteria blooms.

Climate change and the resulting warming of surface waters have also been linking to increases in the frequency of occurrence of cyanobacteria blooms (Gobler, 2020). Given the strong associations between observed cyanobacteria blooms and the aquatic invertebrate communities of wetlands in this study, further research investigating the dynamic relationship between HABs, climate change, and other agricultural stressors on wetland ecology are needed

to help clarify the drivers of this ecosystem dysbiosis and how it might impact aquatic invertebrate communities.

Other water quality parameters were also found to have significant associations with aquatic invertebrate community endpoints, including total nitrogen, which associated with declines in Shannon's diversity, richness, and abundance of multiple invertebrate taxa, as well as an increase in Berger-Parker dominance. Nutrient loading from agriculture has previously been demonstrated to impact aquatic invertebrate communities (Chambers et al., 2006). However, it is unclear whether or not agricultural activities were responsible for the nitrogen levels detected in wetlands in the current study. Agricultural disturbance in the forms of vegetation disturbance and reduced total zone width were not found to have an impact on nitrogen levels detected in wetlands. However, given the history of nutrient loading to aquatic systems from agriculture (Boesch et al., 2001; Withers et al., 2014) and the deleterious associated effects of increased nitrogen on PPR wetland health demonstrated here, it is plausible that agriculture could pose a threat to PPR wetlands through nutrient loading.

In addition to total nitrogen, other water quality parameters including pH, total phosphorus, NPOC, and conductivity were significant in their association with aquatic invertebrate communities in linear models and multivariate analyses. Previous studies have found nutrient and water quality variables such as those discussed in the current study to be significant in their effect on aquatic invertebrate communities in streams (Brett et al., 2017; Camargo & Alonso, 2006; Chambers et al., 2006; Chambers et al., 2012; Clements & Kotalik, 2016). Wetlands of the PPR differ from streams in their dynamic fluctuations in water level and in what would be considered poor water quality parameters in a stream, including high salinity, elevated nutrients, and periods of low dissolved oxygen. As a result, the aquatic invertebrates that inhabit these wetlands are those that can tolerate these harsh conditions (Batzer et al., 1999). While PPR wetland aquatic invertebrate communities are known to comprise more tolerant taxa, this study demonstrated that variation in nutrient and water quality parameters are still extremely important in shaping the composition and dynamics of these communities. The findings of this study also demonstrate that well established aquatic invertebrate biotic indices such as richness, Shannon's Diversity, and Berger-Parker Dominance can serve as useful endpoints in wetland biomonitoring for the effects of excess nutrients.

The occurrence of cyanobacteria blooms and variations in nutrient and other water quality parameters, although not expected, were found to be important in shaping aquatic invertebrate communities of wetlands, However, the extent to which these parameters were influenced by agriculture was not the focus of this study. By contrast, the toxicity of pesticide mixtures quantified in wetlands (PTI) appears to reflect agricultural activity around wetlands and excess nutrients that foster cyanobacteria blooms are likely to co-occur. Although chronic PTIs were not retained or found to be significant in as many aquatic invertebrate models compared to the occurrence of cyanobacteria blooms, chronic PTI was significant in its association with multiple aquatic invertebrate community endpoints. Chronic PTI was significantly associated with a decline in total and relative insect abundance and an increase in relative snail abundance. In other words, wetlands that were contaminated with more toxic mixtures of pesticides supported fewer insects resulting in greater abundance of snails relative to the rest of the macroinvertebrate community in these wetlands.

This observed PTI associated decline in insect abundance builds on the findings from previous publications. Studies conducted in wetlands have demonstrated that field relevant concentrations of neonicotinoids reduce emergence of aquatic insects (Cavallaro et al., 2018, 2019) as well as the biomass of aquatic invertebrates (Schepker et al., 2020). Pesticide associated declines in aquatic insect abundance could explain the reduced insect emergence and invertebrate biomass associated with neonicotinoid concentrations in these previous studies.

The simultaneous PTI associated increase in relative snail abundance is likely a result of the relative tolerance of snails to organic contaminants (von der Ohe & Liess, 2004). Contamination of aquatic systems with neonicotinoids has recently been linked to increased occurrence and relative abundance of certain snails due to their relatively higher tolerance to these pesticides (Becker et al., 2020).

The chronic PTI associated changes in total abundance of certain taxa observed in this study did not have a significant effect on any of the routine biotic indices examined in this study (richness, Shannon's diversity, Shannon's evenness, or Berger-Parker dominance). These findings suggest that when conducting aquatic invertebrate biomonitoring in PPR wetlands, routine biotic indices and absolute abundance are not the most sensitive indicators of ecosystem changes caused by pesticides and are likely insufficient in detecting ecosystem changes occurring in wetlands contaminated with the levels of pesticides reported in this study. Relative

insect taxa and notably the proportional increase in snail abundance could serve as more sensitive endpoints for the biomonitoring of wetland pesticide contamination.

In addition to potentially serving as useful wetland biomonitoring endpoints, the chronic PTI associated changes in relative abundance of insects and snails likely have considerable implications for ecosystem function. Chronic PTI was significantly associated with a shift in relative functional feeding group abundance, which is likely a result of the observed chronic PTI associated shift in relative abundance of insects and snails. This shift in the aquatic invertebrate community could impact the balance of these ecosystems, compromising the many ecosystem services wetlands provide such as nutrient processing and production of food sources for aerial insectivore birds (Hallmann et al., 2014).

Pesticide induced shifts in aquatic invertebrate taxa composition can also influence the spread of diseases. Due to the relatively high insecticide tolerance of many snail taxa relative to other aquatic invertebrates, a study in Kenya found that pesticide contaminated aquatic systems were more likely to have greater dominance of snails (Becker et al., 2020). The planorbidae snails occurred with greater frequency and in greater relative abundance in these polluted systems is concerning as they are intermediate hosts of the flatworm *Schistosoma,* which causes schistosomiasis in humans. While *Schistosoma* does not occur in Saskatchewan, the findings by Becker et al. (2020) highlight the unforeseen impacts that pesticide pollution and ecosystem disruptions can have on human and ecosystem health.

2.6 Conclusions

Wetlands of the PPR are impacted by multiple agricultural stressors including physical disturbance of vegetation and pesticide contamination. The findings of this study indicate that wetland vegetation zones around agriculture imbedded PPR wetlands in Saskatchewan are highly degraded and insufficient in protecting wetlands from pesticide contamination. While it remains unclear whether wetland water quality is impacted by the degree of agricultural disturbance of wetland vegetation, this vegetation disturbance was significantly associated a decline in aquatic invertebrate richness as well as a decline in abundance of some beneficial insects. Water quality was also associated with multiple aspects of wetland health measured through changes in aquatic invertebrate communities. The occurrence of cyanobacteria blooms, predicted toxicity of

pesticide mixtures in wetlands, and increases in nutrients were associated with negative effects to aquatic invertebrate communities in this study.

In summary, this study found that vegetation disturbance and declines in water quality of agriculturally impacted wetlands were associated with negative effects to aquatic invertebrate communities. Aquatic invertebrate communities serve as an indicator of ecosystem health and are an important resource to surrounding ecosystems. This study demonstrated the negative ecosystem health outcomes associated with agricultural stressors in PPR wetlands, a valuable resource that has historically been understudied and degraded.

Chapter 3: Efficacy of Planting Perennial Buffers for Enhancing Wetland Health

3.1 Abstract

Vegetated buffers have been advocated as a tool in mitigating pesticide and nutrient contamination of surface waters, but have yet to be fully researched for their efficacy in protecting Prairie Pothole Region (PPR) wetland health impacted by agricultural activities. Here I examined the efficacy of multiple unique configurations of vegetated buffers (perennial buffers) implemented through producer incentive programs. A total of 17 wetlands on private lands in Saskatchewan with recently planted perennial buffers were compared to 9 wetlands without perennial buffers to assess the concentrations of pesticides and nutrients, other water quality parameters, in relation to the composition of the aquatic invertebrate community. The goal was to evaluate if the presence of planted perennial buffers reduced pesticide and nutrient contamination and improved wetland health. The presence of perennial buffers alone did not significantly reduce pesticide contamination or improve water quality and all wetlands with perennial buffers, even those that were fully surrounded, were contaminated with at least one pesticide. However, fully protected wetlands, those that were completely surrounded by perennial buffers and/or other natural vegetation area were found to have significantly lower insecticide concentrations ($p = 0.001$), chronic Pesticide Toxicity Indices (PTIs) ($p < 0.001$), and total phosphorus ($p = 0.005$). Additionally, increasing the degree of surroundedness of a wetland by planted buffers and/or other natural vegetation was also associated with lower pesticide concentrations ($p = 0.022$) and PTIs ($p < 0.010$).

Perennial buffers consisting of planted forages or other natural vegetation areas enhanced the aquatic invertebrate community. Wetlands with perennial buffers had 32% greater macroinvertebrate abundance on average compared to those without buffers ($p = 0.027$). Wetlands that were fully protected by perennial buffers and/or additional natural vegetation had 50% greater macroinvertebrate abundance on average compared to those without this protection ($p = 0.001$). Perennial buffers were also associated with greater zooplankton ($p = 0.005$) and insect abundance ($p = 0.039$) in wetlands. The results of this study demonstrate that perennial vegetation plantings can effectively reduce, but not eliminate, pesticide and nutrient runoff into wetlands. Planting perennial buffers while also preserving natural vegetation can further enhance aquatic invertebrate production which suggests that programs that incentivize perennial plantings around PPR wetlands may help mitigate the effects of agriculture on aquatic ecosystems and enhance aquatic biodiversity.

3.2 Introduction

Agriculture poses a serious threat to global water quality (Holden et al., 2017; Moss, 2008; United Nations Environmental Programme, 2016). Chemical and manure fertilizers leach excess nutrients into surface and ground waters, causing excess algal growth, deoxygenation, and potentially harmful algal blooms (Howarth et al., 2000; Mendivil-Garcia et al., 2020; Ongley & Nations, 1996). Pesticides carried by runoff contaminate water bodies, posing risks to aquatic life (Becker et al., 2020; Beketov et al., 2013; Berenzen et al., 2005; Liess & von der Ohe, 2005). Meanwhile, tillage of cropland promotes erosion and transport of potentially contaminated sediment into water bodies, increasing turbidity and exacerbating contamination of water bodies with excess nutrients and pesticides (Issaka & Ashraf, 2017; Ongley & Nations, 1996; Van Oost et al., 2009).

A number of mitigation strategies have been researched and promoted as solutions to the complex issue of balancing the need for agriculture with the threats agriculture poses to aquatic ecosystems. Integrated Pest Management (IPM) can reduce pesticide applications and consequently reduce pesticide contamination of aquatic systems (Scott et al., 1999). Another solution that has been widely promoted in agriculture is the implementation of vegetated buffers. Vegetated buffers can consist of different perennial plant communities in various configurations. These buffers are implemented in agricultural fields by taking an area of cropland out of production to establish this perennial vegetation. While vegetation buffers are highly variable in their implementation and reported outcomes, they have proven to be effective in mitigating agricultural runoff contaminated with nutrients and pesticides (Prosser et al., 2020).

Wetlands in Canada's PPR provide many ecosystem services including groundwater recharge (LaBaugh et al., 1998), flood and drought mitigation (Huel, 2000; Woo & Rowsell, 1993), water purification (Johnston, 1991; Pomeroy et al., 2005), carbon sequestration (Badiou et al., 2011), and support of biodiversity (Doherty et al., 2018; Huel, 2000; Janke et al., 2017, 2019; Morrison et al., 2001; National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). Due to intensive agricultural activities in the PPR, up to 89% of wetlands have already been lost due to drainage (Doherty et al., 2018), and 95% of the remaining wetlands in Saskatchewan have their margins physically disturbed or manipulated due to agriculture (Bartzen et al., 2010). In addition to the physical impacts of agriculture, PPR wetlands also face many of the same agricultural threats to water quality as other surface waters around the globe.

Agricultural activities in the PPR can affect nutrient and water quality dynamics (Detenbeck et al., 2002), and runoff from surrounding farmland frequently contaminates PPR wetlands with neonicotinoid insecticides (Main et al., 2014).

Neonicotinoids are prophylactically used in seed treatments of many crops including canola, leading to the widespread use and distribution of these insecticides. In 2015, 229 metric tonnes of insecticide were applied as canola seed treatments in the PPR of Canada (Malaj et al., 2020). Pesticide contamination of PPR wetlands, specifically by neonicotinoid insecticides, has been linked to shifts in emergent aquatic insect community dynamics and reduced abundance, biomass and altered timing of emerging insects (Cavallaro et al., 2018, 2019). Some water quality parameters of wetlands, such as conductivity and total nitrogen, are strong predictors of aquatic invertebrate community composition (Spieles & Mitsch, 2000). Additionally, physical disturbances to wetlands through vegetation removal have been linked to lower emergent insect diversity (Cavallaro et al., 2019). Aquatic invertebrates have long been used for biomonitoring of aquatic lotic and lentic systems (Hodkinson & Jackson, 2005), and the findings in Chapter 2 of this thesis demonstrate the utility of wetland aquatic invertebrate communities to serve as integrative bioindicators of pesticide, nutrient, and physical disturbance stressors.

Although vegetated buffers have been widely studied for their mitigation of pesticide and nutrient runoff into surface waters (Prosser et al., 2020), relatively little of this research has been conducted on wetlands (Aguiar et al., 2015; Main et al., 2015; Moore et al., 2014). One study found that natural wetland vegetation in the outer zones of wetlands can reduce neonicotinoid contamination of PPR wetlands (Main et al., 2015), but researchers have yet to study whether implemented perennial buffers around wetlands can effectively reduce contaminants in runoff. Despite the lack of scientific backing, recommended guidelines suggest maintaining vegetation buffers of at least 10 to 30 meters between cropland and the edge of PPR wetlands (Huel, 2000). In efforts to conserve PPR wetland habitat, Ducks Unlimited Canada (DUC) and Alternative Land Use Canada (ALUS) have worked with producers in Saskatchewan to convert strips of cropland surrounding wetlands to perennial forage. While the specific goal of these programs was not necessarily to reduce pesticide or nutrient runoff, the perennial vegetation plantings are perceived to act as a buffer, mitigating the negative effects of agriculture on wetlands.

Perennial vegetation plantings (perennial buffers) could prove to be an effective tool for mitigating contaminated runoff from agricultural fields and reducing contamination of PPR

wetlands with pesticides and excess nutrients but little scientific guidance exists on their design and efficacy. Additionally, aquatic invertebrate biomonitoring could be an effective integrative tool for assessing the influence of agricultural stressors and the degree to which perennial buffers can improve wetland health. The objectives of this study were to a) examine the relationships between perennial buffers and pesticide and nutrient contamination in PPR wetlands of Saskatchewan and b) assess if and how the aquatic invertebrate community responds to the implementation of perennial buffers. I hypothesized that perennial buffers would be associated with reduced concentrations of pesticides and nutrients. I also hypothesized that the presence of intact or fully surrounded perennial buffers would be associated with significant improvements in aquatic invertebrate community metrics.

3.3 Methods

3.3.1 Study Wetlands Seeded with Perennial Buffers

In 2019, six privately owned agricultural fields were selected for this study in the PPR within 65 km of Yorkton, Saskatchewan. Each field had an established DUC or ALUS perennial vegetation planting (perennial buffer) on them seeded at least 1 year before. Each farmer had control over the particular perennial seed mix planted as well as the configuration and placement of their planting. All perennial vegetation plantings consisted of forage mixes containing grass and alfalfa that were established between 2015 and 2018. Although similar in their programs, DUC's mission is to conserve wetlands and surrounding areas to support waterfowl populations, while ALUS aims at sustaining wildlife and agriculture through enhancing ecosystem services on agricultural land. Both DUC and ALUS are non-governmental organizations (NGOs) that provide incentive programs that compensate farmers for converting cropland to perennial forage on fields that contain wetlands.

Each of the six study fields contained multiple semi permanent or permanent wetlands, with one or more wetlands fully or partially surrounded by perennial vegetation planting (perennial buffer: present), and at least one control wetland which was not surrounded by perennial vegetation planting to any degree (perennial buffer: absent). These six fields, each containing wetlands with and without perennial buffers, allowed for a paired study design with 9 wetlands without perennial buffers and 17 wetlands with perennial buffers (Table 3.1 & Figure

3.1). All wetlands were fishless. Study fields were primarily planted with wheat or canola, with the exception of one field planted with alfalfa.

3.3.2 Quantification of Perennial Plantings: Treatment, Protection, and Percent Protection

The variable "perennial buffer" was used to categorize wetlands with a "perennial buffer absent" (ie. wetlands that are not surrounded by any amount of planted perennial vegetation) and those with a "perennial buffer present" (ie. wetlands that are fully or partially surrounded by planted perennial vegetation) (Table 3.1). However, some study wetlands with and without perennial planted buffers were partially surrounded by natural vegetation area such as grass field margin. Additionally, some study wetlands with perennial buffers were incomplete and had one or more edges adjacent to cropland (Figure 3.1). Since both the perennial planted buffers and natural vegetation areas surrounding wetlands could influence pesticide runoff and contamination, a second categorical variable, "protection" was created. The variable protection was used to categorize wetlands that are "protected" (ie. fully surrounded by perennial vegetation planting and/or natural vegetation area) and "unprotected" (ie. at least 1 edge exposed to cropland) (Table 3.1 and Figure 3.1).

To better understand how wetlands were impacted by surrounding land use, the degree to which study wetlands were surrounded by perennial vegetation planting and natural area was measured as a percentage of the wetland edge (percent perennial and percent natural). These percent surrounding land cover parameters were approximated during on-ground wetland assessments and informed by aerial maps provided by DUC and ALUS. The sum of percent perennial and percent natural was used to calculate the continuous variable "percent protection" (Table 3.1. Eqn. 3.1).Buffer widths were measured at multiple points around each wetland with a perennial buffer, however the sample size of this study was insufficient to examine this buffer width variable in any statistical analysis.

$$\textit{Percent protected} = \textit{percent perennial} + \textit{percent natural} \qquad \text{(Eqn. 3.1)}$$

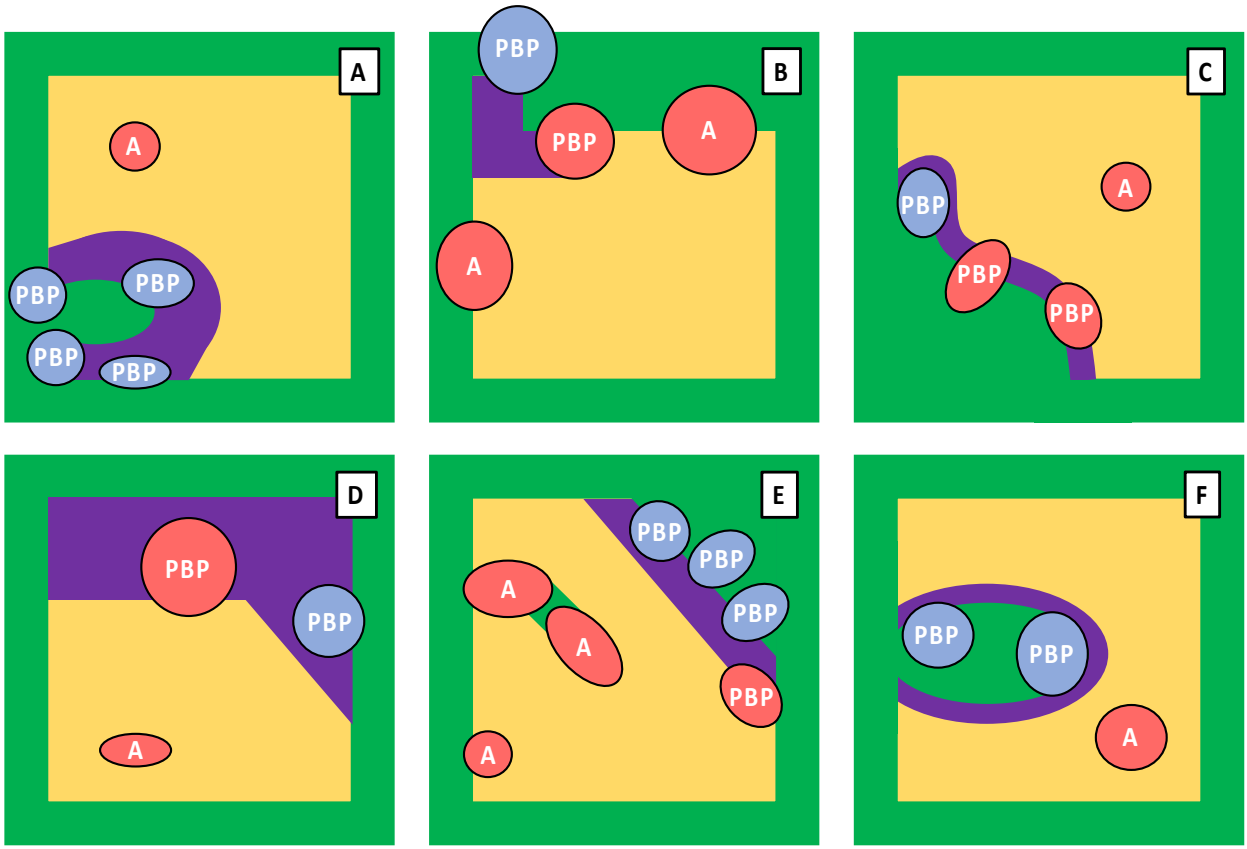

Figure 3.1. Diagram of the 26 study wetlands within the six study fields (A-F) located in the PPR of Saskatchewan (not drawn to scale). Each field contains cropland (yellow), field margin and natural vegetation area (green), and perennial vegetation planting (purple). The 17 wetlands with perennial buffers present "PBP" are to some degree surrounded by perennial vegetation plantings while the 9 wetlands with perennial buffers absent "A" are not surrounded by perennial vegetation plantings to any degree. The 12 "protected" wetlands that are completely surrounded by perennial vegetation plantings and/or natural vegetation area are shown in blue while the 14 "unprotected" wetlands that are to some degree surrounded by crop are shown in pink. Perennial and protection variables in this diagram are summarized in Table 3.1.

Table 3.1. Summary of perennial and protection variables used to categorize the 26 study wetlands located on six agricultural fields in Saskatchewan. Description of categories matches visual color coding from Figure 3.1.

Perennial or Protection Variable	Categories	Description	Number of Wetlands
Perennial Buffer	absent (A)	not surrounded by perennial buffer to any degree	9
	present (PBP)	surrounded by perennial buffer to some degree	17
Protection	unprotected (pink)	not fully surrounded by perennial buffer and/or other natural vegetation area	14
	protected (blue)	fully surrounded by perennial buffer and/or other natural vegetation area	12
Percent Protection	NA (continuous variable)	percentage of the wetland edge surrounded by perennial and/or natural vegetation area	26

3.3.3 Other Wetland Variables Recorded

Additional wetland variables were recorded in order to account for the natural variation among study wetlands. Wetland surface area (area) was measured using aerial maps provided by DUC and ALUS. Wetland depth was recorded for each study wetland as being greater than or less than 1 meter.

3.3.4 Wetland Water Sampling

Water samples were collected from each study wetland at multiple points throughout the summer, to be analyzed subsequently for pesticides and water quality parameters. All water samples were collected by wading into the central water body of a wetland and filling containers dipped below the water surface, in front of any sediment that might be disturbed by carefully entering the wetland. Subsurface water samples were collected in 2-L amber glass bottles for pesticide analysis (see section 3.3.5). In addition, a 1-L high-density polyethylene jug was filled with water for more general water quality analysis including that of nutrients (see section 3.3.6). Samples were stored in coolers in the field, later transferred to refrigerators at 4^{o}C, and extracted

within 4 weeks of collection for pesticides and 48 hours for nutrients. Holding time limits as well as dark and refrigerated sample storage conditions were maintained to reduce pesticide degradation and metabolism as a result of UV light exposure or microbial growth (Acero et al., 2019; Bansal, 2012).

Water samples for pesticide analysis were collected from each wetland at two sampling periods throughout the growing season of 2019: early May and late June. The May sampling time was chosen to capture contamination from spring runoff containing pesticides from the previous year. By early May, wetlands had just thawed, and farmers were beginning to seed fields. The June sampling period was chosen to capture contamination from field applications in the current year. Sampling was not conducted in July of 2019 due to dry conditions which caused many wetlands to no longer contain water. Water samples for water quality measurements were collected once from each wetland in late June, corresponding with June sampling for pesticide analysis.

3.3.5 Pesticide Analysis of Water Samples

Water samples were analyzed for four neonicotinoid insecticides (imidacloprid, thiamethoxam, clothianidin, acetamiprid) and six other systemic insecticides (chlorantraniliprole, cyantraniliprole, flonicamid, flubendiamide, flupyradifurone, and sulfoxaflor) at the National Hydrology Research Center, Environment and Climate Change Canada in Saskatoon, Saskatchewan. Analytical methods for LC/MS/MS were adapted from previously published methods (Xie et al., 2011). Samples with pesticide concentrations (μg/L) below the limit of quantification (LOQ) were treated as non-detections (zeroes) and not assigned a value between zero and the LOQ in order to avoid overestimating total pesticide concentrations. A list of the LOQs and recoveries of spiked samples for all pesticides analyzed can be found in Appendix A and B. Laboratory blanks were all below detection and sample data were not recovery corrected. Total pesticide concentrations of the 10 pesticides were calculated as the sum of all quantified pesticides in a wetland in a given sampling period.

3.3.6 Acute and Chronic Pesticide Toxicity Index (PTI) Calculation

The pesticides detected in wetlands have a range of potential toxicities to aquatic life. To assess the relative toxicity of each pesticide mixture detected in study wetlands, Pesticide

Toxicity Indices (PTIs) were calculated by summing the ratios of individual pesticide concentrations detected relative to that chemical's hazard concentration (HC_5) protective of 95% of the aquatic taxa drawn from an acute or chronic species sensitivity distribution (SSD) which was populated with published values from aquatic organism toxicity assays (Nowell et al., 2014). In cases where less than 7 values were available for use in SSDs, minimum toxicity values representing the most sensitive published toxicity value were used in the place of an HC_5 (Appendix C). To assess the risk associated with pesticide contamination that the June sampled aquatic invertebrate community was exposed to, acute and chronic PTIs were calculated using the maximum and average concentrations of pesticides detected in wetlands in May and June. PTIs were only calculated when pesticide data were available for both of the sampling periods used for calculation.

$$Acute\ PTI = \sum_{i=1}^{n} \frac{\boldsymbol{maximum\ concentration\ in\ May\ and\ June}}{\boldsymbol{acute\ HC5}} \qquad \text{(Eqn. 3.2)}$$

$$Chronic\ PTI = \sum_{i=1}^{n} \frac{\boldsymbol{mean\ concentration\ for\ May\ and\ June}}{\boldsymbol{chronic\ HC5}} \qquad \text{(Eqn. 3.3)}$$

3.3.7 Nutrient and General Water Quality Analysis of Water Samples

Water samples were analyzed at the Dorset Environmental Science Center, Dorset, Ontario for nutrients and standard water quality parameters including conductivity, pH, total phosphorus (TP), potassium, and total nitrogen (TN).

3.3.8 Aquatic Invertebrate Collection

Aquatic invertebrate samples were collected at the same time as the June water sampling. At this time, wetlands were in a period of peak productivity and insect emergence. Samples were collected using the Canadian Aquatic Biomonitoring Network (CABIN) Protocol for wetlands (Environment and Climate Change Canada, 2018), with a 400 μm mesh D-frame sweep net. In an area of the wetland with emergent and submergent aquatic plants, the net was dipped beneath the water and moved in a zigzag pattern back and forth as the sampler walked forward, moving the net up and down through the water column and tapping the substrate to stir up benthic

invertebrates. This motion was continued through the area of emergent and submergent aquatic plants for 2 minutes.

Aquatic invertebrate samples containing debris and vegetation were allowed to drain in the net before being transferred to 1-L plastic collection sample containers. Samples were preserved with ethanol in the field, adding 95% ethanol to sample containers to achieve approximately 70% ethanol in preserved samples.

3.3.9 Aquatic Invertebrate Subsampling and Identification

Aquatic invertebrate samples were later processed, subsampled, and identified in the laboratory at the University of Saskatchewan using a modified version of the CABIN Laboratory Methods for Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples (Environment and Climate Change Canada, 2014) with a Marchant box (Marchant, 1989) for aquatic invertebrate subsampling.

Samples were first rinsed by gently mixing each sample in a bucket of excess water. Large debris and vegetation were removed from the sample after thoroughly rinsing invertebrates and sediment on the debris/vegetation back into the sample being washed. Samples were then poured through a 400-μm mesh sieve, removing fine sediment from the sample.
Before beginning the process of subsampling, any large invertebrate ≥ 1.5 cm was removed and sorted to order to be identified later. This amendment to the original CABIN protocol was included due to the presence of large invertebrates such as Lymnaeidae and Belistomatidae that were not effectively subsampled using Marchant box cells. Additionally, by removing invertebrates ≥ 1.5 cm, large soft bodied invertebrates such as those from the order Odonata, were less likely to be damaged which would have led to difficulties during later identification.

Rinsed samples were transferred into a 100-cell Marchant box, filling the box with enough water to fill all Marchant box cells. After securing the lid on the Marchant box, the box was inverted, agitated, and quickly inverted again to best distribute invertebrates evenly. A random number generator was then used to select multiple cells from the 10 x 10 grid of 100 Marchant box cells for sorting and later identification. The contents of the first selected cell were then removed and transferred to a petri dish. Working under a dissecting microscope, all invertebrates from the selected cell were sorted into vials containing 70% ethanol by order and tallied. The original CABIN protocol does not include zooplankton, but was amended for the current study by sorting and recording abundance of three zooplankton taxa that were

particularly abundant in our study wetlands (Cladocera, Copepoda, and Ostracoda) from the first randomly selected cell only.

After completion of the first cell, additional cells were randomly selected for sorting and tallying of all macroinvertebrates. Once a cell was started, it was always sorted in entirety. Randomly selected cells were sorted until two criteria were reached: 1) a minimum of 5 cells were sampled and 2) a minimum of 300 *insect* macroinvertebrates had been tallied. The original CABIN protocol requires a minimum of 300 macroinvertebrates, but not necessarily insects. This modification was made to the protocol to ensure that sufficient diversity was captured in wetlands that were heavily dominated by Gastropoda and Amphipoda. In this modified protocol, non-insect taxa were still sorted and tallied as were insect taxa, but only the insect taxa counted toward the 300 macroinvertebrate minimum.

After sorting of invertebrates to order, macroinvertebrates were identified to varying taxonomic levels using a dissecting microscope. Taxonomic keys (Clifford, 1991) as well as consultation with Iain Phillips, an aquatic invertebrate taxonomy specialist at the Water Security Agency, Government of Saskatchewan, Saskatoon, Saskatchewan were used for identification. Most insects were identified to genus, with the exception of Diptera and non-Corixidae Hempitera which were only identified to family.

To calculate estimated abundance of each zooplankton and macroinvertebrate taxa in each sample based on the number of organisms in the subsample, the following equations were used:

$$\textit{Abundance of a given zooplankton taxa} = (\textit{number of organisms in cell 1}) \times 100\ \textit{cells} \quad \text{(Eqn. 3.4)}$$

$$\textit{Abundance of a given macroinvertebrate taxa} = (\textit{number of organisms} \geq \textit{1.5 cm}) + \frac{\text{number of organisms} < 1.5\ \text{cm}}{\textit{number of cells sorted}} \times 100\ \textit{cells} \quad \text{(Eqn. 3.5)}$$

3.3.10 Aquatic Invertebrate Community Indices

Occurrence and abundances of zooplankton and macroinvertebrates measured in wetlands were used to calculate aquatic invertebrate community indices. Richness (*S*) was determined for each wetland as the number of macroinvertebrate taxa identified in each wetland.

Shannon's diversity (*H*) was calculated for each wetland in which p_i is the proportion of *S* made up of the *i*th macroinvertebrate taxa.

$$H = -\sum_{i=1}^{S} p_i \ln p_i \qquad \text{(Eqn. 3.6)}$$

Shannon's evenness (E_H) was calculated for each wetland by dividing *H* by H_{max} (here $H_{max} = \ln S$).

$$E_H = \frac{H}{H_{max}} = \frac{H}{\ln S} \qquad \text{(Eqn. 3.7)}$$

Berger-Parker dominance (*d*) was calculated for each wetland by dividing the number of individuals in the most abundant macroinvertebrate taxa (N_{max}) by the total number of individuals in all macroinvertebrate taxa (*N*).

$$d = \frac{N_{max}}{N} \qquad \text{(Eqn. 3.8)}$$

Hilsenhoff Biotic Index (*HBI*) was calculated for each wetland where x_i is the number of individuals in the *i*th macroinvertebrate taxon, t_i is the tolerance value of the *i*th macroinvertebrate taxon, and *n* is the total number of macroinvertebrates in the sample (Mandaville, 2002).

$$HBI = \frac{\sum x_i t_i}{n} \qquad \text{(Eqn. 3.9)}$$

In addition to the above calculated biotic indices, total abundance of macroinvertebrates, zooplankton, insects, and gastropods were calculated for use as endpoints in linear models.

3.3.11 Statistical Analysis

3.3.11.1 Relationship Between Perennial Buffers and Pesticides and Water Quality

We used linear models to analyze the relationship between the presence of buffers or protection and pesticide concentrations (total pesticide concentration in water sample averaged over multiple sampling periods for each wetland), chronic PTI, and nutrient concentrations including total nitrogen, total phosphorus, and potassium, using data from 26 wetlands on six

agricultural fields sampled during the growing season of 2019. All analyses were performed in R studio version 1.1.456. Due to insufficient water levels during the June sampling period, only 22 wetlands were used in these analyses, with the exception of the total phosphorus analysis which used 18 wetlands due to missing values. Pesticide and water quality parameters were transformed to improve normality and residuals prior to modeling and individual pairs of variables were tested for collinearity ($r < 0.7$) before inclusion in the same model.

Three separate global linear models of each pesticide and water quality parameter were performed to evaluate responses of each of the three perennial or protection variables (perennial buffer, protection, or percent protection). Additional variables were included such as wetland depth, wetland area, crop type, and field as fixed effects. Field could not be modeled as a random effect as this led to overfitting the models. Pearson's r-correlation coefficients of continuous fixed effects were all less than 0.70 (Appendix O & P). Model selection was then performed on global models guided by AICc (Appendix Q) using the "dredge" function in the R package MuMIn (Barton´, 2020). Models with the lowest AICc scores or $\Delta AICc < 2$ were considered as final models, using model averaging for all models with $\Delta AICc < 2$. Only final models or averages of final models found to have lower AICc values than null models were further analyzed for model results.

3.3.11.2 Relationship Between Perennial Buffers and the Aquatic Invertebrate Community

Linear models were also used to analyze the effects of perennial buffers (perennial buffer, protection, or percent protection) on multiple aquatic invertebrate community endpoints (richness, Shannon's Diversity, Shannon's Evenness, Hilsenhoff Biotic Index, and Berger-Parker dominance, abundance of macroinvertebrates, zooplankton, and insects). Due to insufficient water levels for aquatic invertebrate sampling as well one wetland excluded from analysis due to a prominant cyanobacteria bloom, linear models were performed on data from 20 study wetlands.

Three separate global models of each aquatic invertebrate community endpoint were performed containing one of the three perennial or protection variables (perennial buffer, protection, or percent protection) as well as wetland depth, crop type, wetland area, conductivity, pH, potassium, total nitrogen, log(chronic PTI), and field as fixed effects. Pearson's r-correlation

coefficients of continuous fixed effects were all less than 0.70 (Appendix R). Field was not modeled as a random effect as this would have led to overfitting of the models. Model selection was then performed on global models guided by AICc (Appendix S) using methods identical to those described in section 3.3.11.1.

3.4 Results

3.4.1 Effect of Perennial Vegetation Plantings on Pesticides and Water Quality of Wetlands

Water samples taken in May and June from the 26 study wetlands were analyzed for concentrations of 10 current use insecticides. Five different insecticides were detected including four neonicotinoid insecticides and one diamide class neonicotinoid replacement. All but one of the 26 study wetlands were found to contain quantifiable concentrations of insecticides. The neonicotinoid class insecticide thiamethoxam was the most frequently detected insecticide, found in 53.1% of samples (Table 3.2). Other aspects of water quality were assessed in 22 of these wetlands (Table 3.3) in June and PTIs were calculated based on concentrations of pesticides measured in wetlands in May and June relative to their published toxicity values. Acute PTIs ranged from 0 to 0.136 and chronic PTIs ranged from 0 to 8.39, with 13 chronic PTIs exceeding the threshold of 1.0 (59% of wetlands) (Figure 3.2).

Table 3.2. Concentration and detection of neonicotinoid and diamide insecticides quantified in water samples from 26 study wetlands in the PPR of Saskatchewan in May and June of 2019. Concentrations below LOQs (Appendix A) were set at 0.0001 μg/L for calculation of geometric means in this table.

Pesticide Class	Pesticide	Geometric Mean (μg/L)	Concentration Range (μg/L)	Percent Detection
Neonicotinoid Insecticide	Imidacloprid	< 0.001	0 - 0.019	14.3
	Thiamethoxam	0.001	0 - 0.065	53.1
	Clothianidin	< 0.001	0 - 0.023	38.8
	Acetamiprid	< 0.001	0 - 0.013	8.2
Diamide Insecticide	Chlorantraniliprole	0.001	0 - 0.093	42.9
All Insecticides		**0.005**	**0 - 0.158**	**83.7**

Table 3.3. Arithmetic mean and rage for water quality variables measured in 22 wetlands in the PPR of Saskatchewan in June 2019.

Parameter	Mean	Range
Conductivity (μS/cm)	1,036 ± 500	391 – 2,010
pH	8.3 ± 0.8	7.4 – 9.9
*Total Phosphorus (mg/L)	0.25 ± 0.33	0.04 – 1.26
Potassium (mg/L)	30.21 ± 12.73	10.00 – 50.30
Total Nitrogen (mg/L)	2.54 ± 0.75	1.48 – 3.96

*Total phosphorus reported for 18 wetlands due to missing values

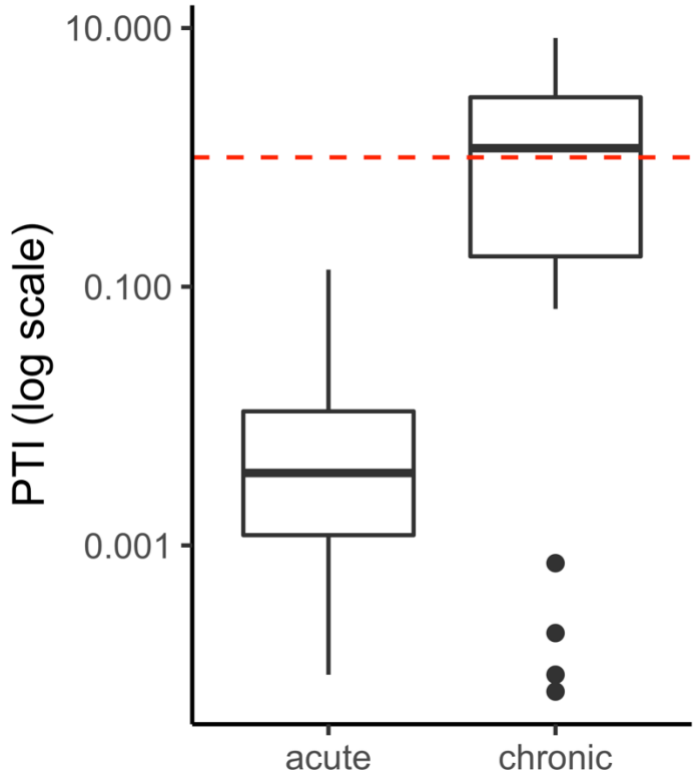

Figure 3.2. Boxplot of acute and chronic Pesticide Toxicity Indices (PTIs) for 22 Saskatchewan wetlands sampled in May and June of 2019. The horizontal red dashed line marks a PTI of 1.0, the risk threshold at which the pesticide mixture has met the HC_5. PTIs higher than 1.0 (red line) indicate increasing risk to aquatic life. PTIs of 0 are represented as 0.0001 on the log scale in this plot.

Linear models of the total pesticide concentration in water samples averaged over multiple sampling periods (pesticide concentration), total nitrogen, total phosphorus, potassium, and chronic PTI followed by AICc based model selection was performed to investigate their relationship with perennial planting or protection variables (Appendix Q). Perennial buffers alone were not found to be significantly associated with any aspect of water quality (Appendix T). However, protection and percent protection were significantly associated with multiple differences in water quality. Protected wetlands and those with greater percent protection had significantly lower pesticide concentrations (Protection: $p = 0.001$ and Percent Protection: $p = 0.022$) (Figure 3.3) and chronic PTIs (Protection: $p < 0.001$ and Percent Protection: $p = 0.010$) (Appendix T). Additionally, protected wetlands were found to have significantly lower concentrations of total phosphorus ($p = 0.005$) (Figure 3.4).

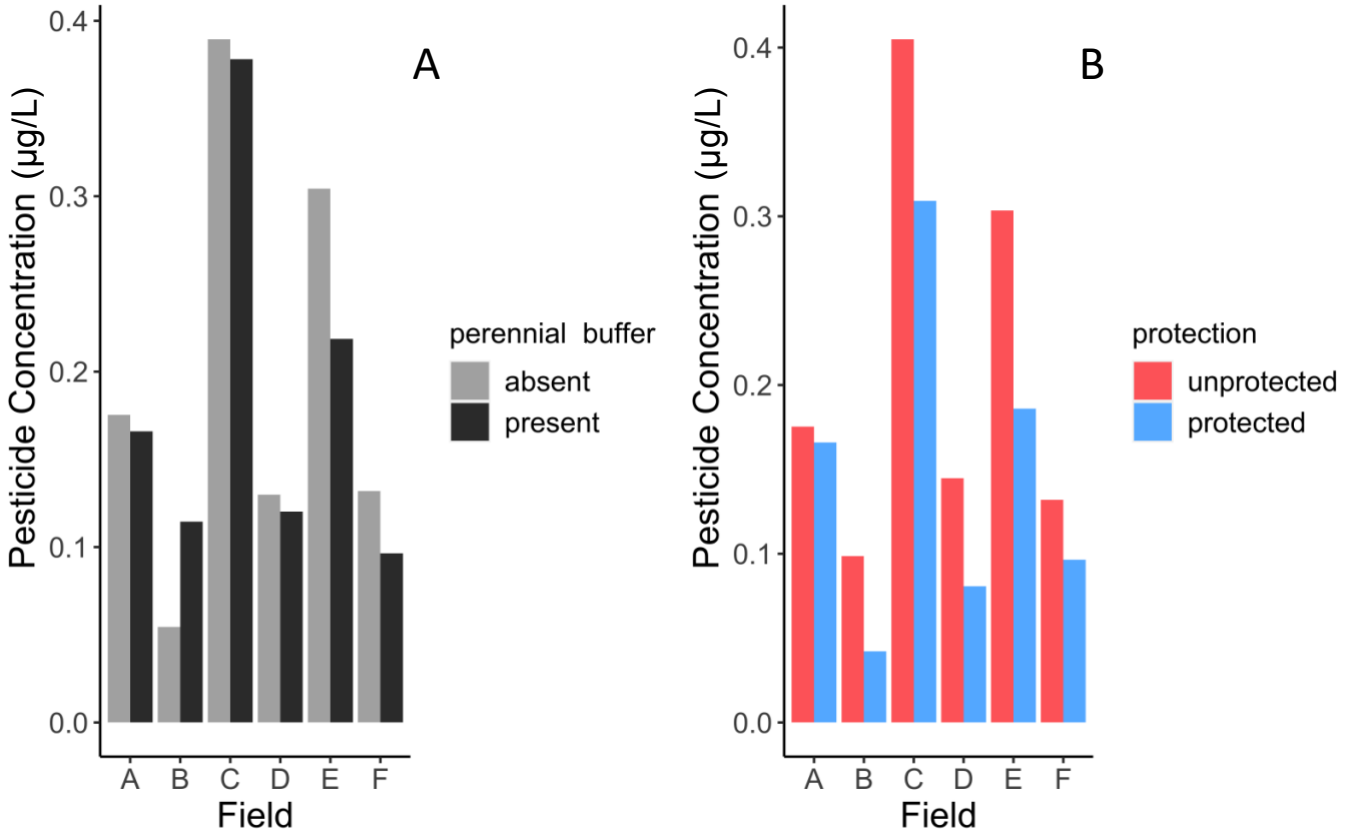

Figure 3.3. Cube root transformed pesticide concentrations (total concentration in sample averaged between sampling points and perennial or protection groups) (µg/L) measured in 26 wetlands located on six fields in Saskatchewan sampled twice during the growing season of 2019. A) Average pesticide concentrations measured in wetlands with perennial buffers absent (grey) and present (black) on each of the six fields (A-F). B) Average pesticide concentrations measured in protected (blue) and unprotected (pink) wetlands on each of the six fields (A-F). In linear models of pesticide concentration measured in 22 of these study wetlands (Appendix Q), protection (B) was a significant predictor of pesticide concentration, with protected wetland having lower concentrations of pesticides ($p = 0.001$). Perennial buffer (A) was not significant ($p > 0.05$) (Appendix T).

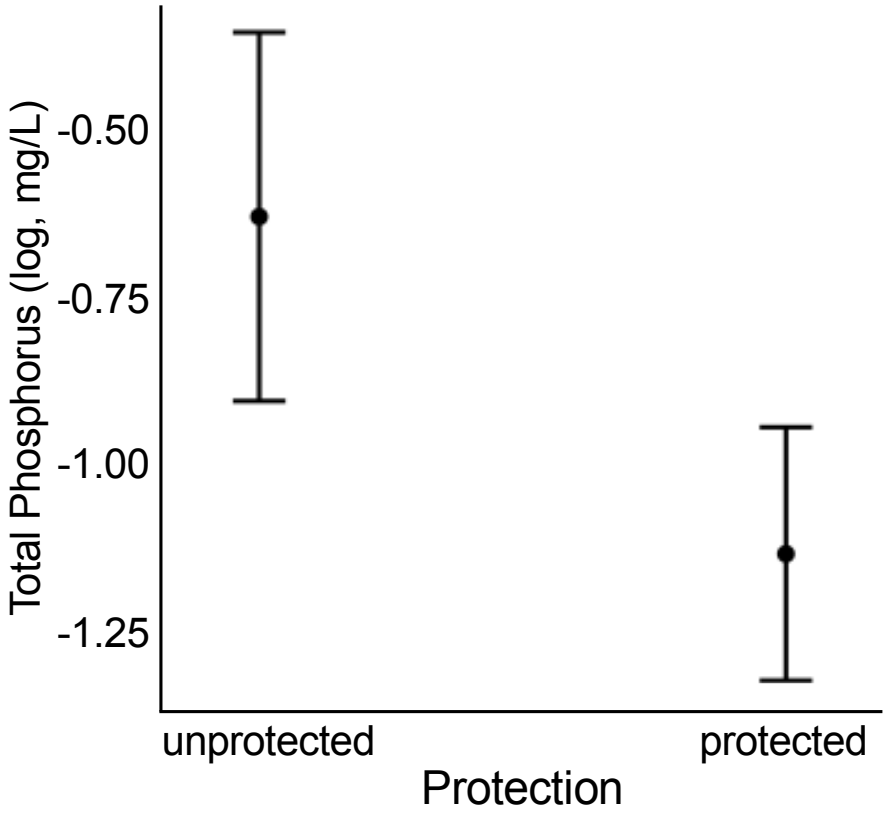

Figure 3.4. Influence of vegetated protection on model predicted means for total phosphorus sampled in 18 wetlands in Saskatchewan in June of 2019. Protected wetlands had significantly lower concentrations of total phosphorus compared to unprotected wetlands ($p = 0.005$) (Appendix T).

Although pesticide concentrations were found to be significantly lower in protected wetlands, all protected wetlands and those with perennial buffers were found to be contaminated with pesticides at quantifiable concentrations. The only wetland with a total pesticide concentration of zero during a sampling period was an unprotected wetland on field B (Figure 3.1) which was 50% surrounded by natural vegetation and 50% surrounded by crop.

3.4.3 Perennial Buffers and other Factors Influencing Aquatic Invertebrate Communities

Aquatic invertebrate community endpoints were calculated for 20 study wetlands sampled in June 2019 (Table 3.4). Linear models of each endpoint were performed using perennial planting or protection variables, environmental variables, and water quality variables followed by AICc based model selection (Appendix S). Perennial plantings and protection

variables were not found to have a significant influence on any of the wetland aquatic invertebrate biotic indices calculated in this study (richness, Shannon's diversity, Shannon's Evenness, Berger Parker Dominance, and Hilsenhoff Biotic Index) (Appendix S). However, perennial and protection variables were significantly associated with greater abundances of organisms in multiple invertebrate groups. Samples from wetlands with perennial buffers had 32% greater macroinvertebrate abundance on average compared to those from wetlands without buffers. Samples from wetlands that were fully protected by perennial buffers and/or additional natural vegetation had 50% greater macroinvertebrate abundance on average compared to those from wetlands without this protection. This greater abundance of macroinvertebrates in samples from wetlands with perennial buffers and protection was significant in linear models (Perennial buffer: $p = 0.027$ and Protection: $p = 0.001$) (Table 3.5 & Figure 3.5). Increasing degree of protection (percent protection) was also associated with greater macroinvertebrate abundance ($p = 0.005$) (Table 3.5). In the linear models of macroinvertebrate abundance containing the predictor variables perennial buffer and percent protection, chronic PTI was associated with lower macroinvertebrate abundance ($p = 0.003$ and $p = 0.023$) (Table 3.5). Presence of perennial buffers around wetlands was also associated with greater zooplankton and insect abundance in wetland samples ($p = 0.005$ and $p = 0.039$) (Table 3.5). Greater percent protection was also associated with greater zooplankton abundance ($p = 0.014$) (Table 3.5).

Table 3.4. Aquatic invertebrate community indices including macroinvertebrate and zooplankton abundance as well and richness, Shannon's diversity, Shannon's evenness, Berger-Parker dominance, and Hilsenhoff Biotic Index for macroinvertebrates collected from 20 Saskatchewan wetlands in June 2019.

Aquatic Invertebrate Biotic Index or Endpoint	**Median (Range)**	**Mean ± SD**
Total Macroinvertebrate Abundance	4,800 (144 – 9,108)	4,949 ± 2151
Total Zooplankton Abundance	14,650 (289 – 45,000)	17,440 ± 11,325
Richness	22 (11 - 38)	22.6 ± 7.6
Hilsenhoff Biotic Index	6.7 (6.1 - 7.0)	6.6 ± 0.2
Shannon's Diversity	1.2 (0.6 - 2.3)	1.3 ± 0.5
Shannon's Evenness	0.4 (0.2 - 0.7)	0.4 ± 0.1
Berger-Parker Dominance	0.7 (0.3 - 0.9)	0.6 ± 0.2

Table 3.5. Linear model parameter estimates for effect of perennial buffer, protection, and other environmental and water quality variables on aquatic invertebrate community responses

(macroinvertebrate, zooplankton and insect abundance) measured in 20 Saskatchewan wetlands (Appendix S). Bolded values indicate statistical significance ($p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***) of parameters. Results are presented only for the models with AICc values less than those of null models (Appendix S).

Response	Perennial or Protection Variable Included in Global Model	Parameter	Estimate ± SE	p-value	
Macroinvertebrate Abundance	perennial buffer	**area**	-1028.9 ± 345.9	**0.006**	**
		perennial buffer: present	1750.2 ± 736.5	**0.027**	*
		log chronic PTI	-1094.2 ± 339.4	**0.003**	**
		depth: shallow	-1267.8 ± 677.6	0.084	
		conductivity	-591.2 ± 349.1	0.119	
	protection	**area**	-890.7 ± 346.3	**0.017**	*
		depth: shallow	-1447.1 ± 651.3	**0.04**	*
		protection: protected	2618.7 ± 698.6	**0.001**	***
	percent protection	**area**	-921.9 ± 362.7	**0.019**	*
		percent protection	938.2 ± 407	**0.032**	*
		log chronic PTI	-980.3 ± 401.3	**0.023**	*
		depth: shallow	-1437 ± 715.8	0.063	
		conductivity	-595.3 ± 363.3	0.132	
Zooplankton Abundance	perennial buffer	**area**	-6700 ± 2772	**0.022**	*
		depth: shallow	-12542 ± 5562	**0.034**	*
		potassium	6177 ± 2955	0.053	
		perennial buffer: present	12652 ± 4172	**0.005**	**
		total nitrogen	-3319 ± 2039	0.135	
		pH	-3437 ± 2154	0.145	
	percent protection	area	-4483 ± 2526	0.095	
		percent protection	6538 ± 2495	**0.014**	*
		conductivity	-6645 ± 3377	0.062	
		crop type: other	-7369 ± 7904	0.392	
		crop type: wheat	-20425 ± 7779	**0.016**	*
		pH	-3529 ± 2537	0.196	
		depth: shallow	-11895 ± 7953	0.15	
		potassium	6514 ± 3373	0.074	
		total nitrogen	-3028 ± 2368	0.236	

		conductivity	-1100.4 ± 468.2	**0.029**	*
		potassium	-762.8 ± 407.9	0.084	
Insect Abundance	perennial buffer	**perennial buffer: present**	2042.4 ± 918.5	**0.039**	*
		area	-719.6 ± 420.5	0.114	
		depth: shallow	-1452.6 ± 891.6	0.13	

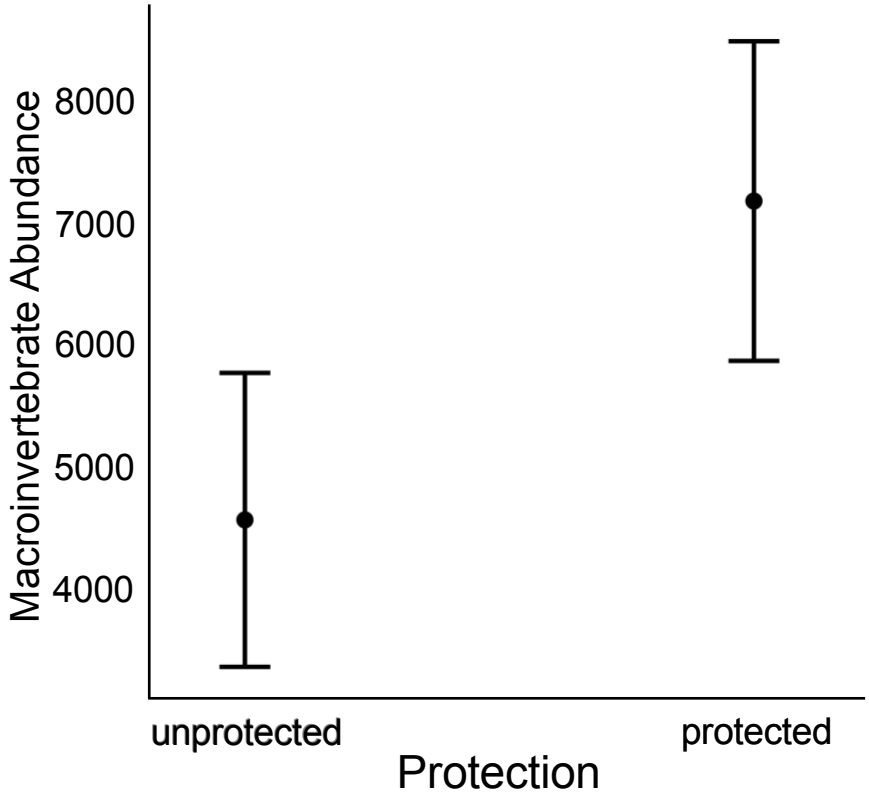

Figure 3.5. Influence of vegetated protection on the model predicted means for total macroinvertebrate abundance sampled in 20 Saskatchewan wetlands in June of 2019. Protected wetlands contained significantly greater macroinvertebrate abundance compared to unprotected wetlands (p = 0.001) (Table 3.5).

3.5 Discussion

Perennial vegetation buffers can be effective tools for reducing pesticide and nutrient contamination of water bodies (Prosser et al., 2020) and increasing agroecosystem biodiversity (Lind et al., 2019; Schulte et al., 2017). The findings presented in Chapter 2 suggest that natural wetland vegetation in the vegetation zones of PPR wetlands might not be sufficient at mitigating pesticide runoff into PPR wetlands. Here I explored whether or not implementation of perennial vegetation plantings around wetlands could act as buffers, mitigating pesticide and nutrient runoff and improving wetland health. I hypothesized that pesticide and nutrient contamination would be significantly reduced by perennial vegetation plantings. Presence of a perennial buffer alone were not found to significantly reduce contamination of wetlands with pesticides or nutrients. However, fully protected wetlands and those with greater percent protection were found to have significantly lower pesticide concentrations and chronic PTIs. Protected wetlands were also found to have significantly lower concentrations of total phosphorus and were associated with higher macroinvertebrate, zooplankton and insect abundance

The finding that the presence of perennial buffers alone did not have a significant effect on pesticide or nutrient concentrations in wetlands is likely a result of where farmers implemented perennial plantings. Crop area converted to perennial plantings was often along natural field margins or other areas of natural vegetation. This resulted in many wetlands being partially surrounded by perennial plantings and partially surrounded by natural vegetation areas. Multiple wetlands were also left only partially protected by perennial plantings with the majority of their wetland edge surrounded by crop. For these reasons, the vegetation protection of wetlands on study fields was often decoupled from the perennial buffer treatment. This is likely why the categorical variable protection was a better predictor of pesticide concentration, PTI, and total phosphorus compared to presence of perennial buffers. This suggests that it is not enough for a wetland to be partially surrounded by a perennial buffer for the mitigation of pesticide and nutrient contamination. If a farmer or land manager wishes to reduce pesticide and nutrient runoff into their wetlands, these results suggest that they could implement perennial plantings so that wetlands are completely surrounded by the plantings, in addition to whatever natural vegetation already exists around wetlands.

Although wetlands surrounded by vegetation protection contained less pesticide and nutrient contamination, all study wetlands with perennial buffers as well as those fully protected

by vegetation were found to be contaminated with quantifiable concentrations of pesticides. This suggests that even if a wetland is fully surrounded by a perennial buffer, this will not necessarily eliminate pesticide contaminate in the protected wetland. The observed pesticide contamination in all perennial buffer and protected wetlands is evidence that there might be additional factors that are not accounted for in this study that are influencing pesticide contamination of wetlands. For example, the vegetation protection investigated in this study could have been insufficient at mitigating aerially transported pesticides resulting from spray drift (Tsai et al., 2005) and seed treatment dust (Devarrewaere et al., 2018). Regardless of what factors may be contributing to the pesticide contamination measured in this study, if one's goal is to completely prevent the contamination of wetlands with pesticides, one would either need to implement vegetation buffers beyond what were investigated in this study or prevent pesticide contamination in other ways such as reducing the amount of pesticide applied to crops, perhaps by using IPM practices (Scott et al., 1999).

We also hypothesized that the perennial vegetation plantings would have a significant effect on aquatic invertebrate communities in wetlands. Perennial plantings and protection variables were not found to have a significant effect on any of the wetland aquatic invertebrate biotic indices calculated in this study (richness, Shannon's diversity, Shannon's Evenness, Berger Parker Dominance, and Hilsenhoff Biotic Index). However, perennial and protection variables were associated with significant increases in total abundance of macroinvertebrates, zooplankton, and insects.

While perennial buffers and protection of study wetlands were associated with increased abundance of organisms within invertebrate groups, greater predicted pesticide toxicity (chronic PTI) of these same study wetlands was associated with lower macroinvertebrate abundance. This result is consistent with the findings in Chapter 2 of this study and suggests that in addition to mitigating pesticide contamination, vegetation protection of wetlands could also help improve aquatic invertebrate production through enhanced wetland health and condition.

One of the goals of perennial buffers is to reduce agricultural disturbances and their effects on the aquatic invertebrate community. Studies looking at the effects of vegetation disturbance on wetlands have found that agricultural vegetation disturbance and mowing of vegetation around wetlands increase emergent insect abundance (Cavallaro et al., 2019) and benthic invertebrate abundance (De Szalay et al., 1996) respectively. By contrast, the present

study found increased invertebrate abundance in perennial and protected wetlands, which were assumed to be protected from vegetation disturbance. Although perennial plantings examined in this study generally result in less disturbance compared to the usual annual cropping of canola and wheat, these perennial plantings were still managed, resulting in some level of vegetation disturbance in the form of mowing and haying of the perennial forage. The greater abundance of invertebrates observed in perennial and protected wetlands could be a result of disturbance in the form of mowing, similar to what was reported by De Szalay et al (1996). Alternatively, the aquatic invertebrate communities of wetlands in the current study could be responding to the increased amount of vegetation habitat provided by perennial buffers. One study found that vegetation surrounding wetlands provided important habitat for Odontata which were more abundant in wetlands with more vegetation habitat (Foote & Hornung, 2005). Therefore, the greater invertebrate abundance observed in perennial and protected wetlands could be a result of the terrestrial vegetation habitat provided by perennial buffers.

While fewer studies have researched factors influencing zooplankton communities in wetlands, some studies have found zooplankton communities to be responsive to differences in vegetation. Increases in zooplankton abundance were reported to be highly correlated with the presence of submerged and emergent vegetation (Gebrehiwot et al., 2017; Norlin et al., 2005). A study examining effects of land use and vegetation buffers on zooplankton in shallow lakes found that agriculture surrounded lakes with vegetation buffers had greater zooplankton richness compared to lakes with no buffers (Dodson et al., 2005). The findings by Dodson et al. (2005) demonstrate a link between zooplankton communities and surrounding land use. Although it is unclear what mediated the effects of land use on zooplankton communities, the greater zooplankton abundance observed in wetlands with perennial buffers in the current study could be mediated by similar vegetation buffer effects that were linked to greater zooplankton richness by Dodson et al. (2005).

Wetland invertebrates are an important food source for many animals in and around wetlands. The diet of the northern shoveler (*Spatula clypeata*), a waterfowl species that migrates through the PPR of Saskatchewan, is largely comprised of zooplankton (Euliss et al., 1991). Emergent insects are a vital food source for aerial insectivore birds, populations of which have been in decline (Manning & Sullivan, 2021). Non emergent insects and other macroinvertebrates are important food sources for waterfowl like blue-winged teal (*Spatula discors*) (Swanson et al.,

1974). Waterfowl hens in particular rely on the calcium in snails and crustaceans for egg production (Eldridge, 1990). Protection of PPR wetlands with perennial vegetation plantings and the associated increases observed in wetland invertebrate abundances could help support critical wildlife such as these migratory birds.

3.6 Conclusions

Implementation of vegetated buffers has been proven to be effective at mitigating pesticide contaminated runoff in a number of agricultural scenarios. In this study, DUC and ALUS perennial buffer incentive programs implemented around Saskatchewan PPR wetlands were examined for their efficacy in mitigating pesticide and nutrient runoff and improving wetland health. Although this study was not able to find a direct relationship between the presence of perennial buffers and reductions in pesticide or nutrient contamination, we did find that wetlands that were *fully surrounded* by perennial buffers or other natural vegetation had significantly lower pesticide and nutrient concentrations. Perennial buffers and additional vegetation protection from natural vegetation areas were also associated with greater abundances of aquatic invertebrates of multiple taxa which serve as important food sources for wildlife. Although vegetation protection was not found to completely eliminate pesticide contamination of wetlands, the observed greater abundance of aquatic invertebrates in protected wetlands could help offset the potential negative effects of low pesticide contamination. The findings of this study could help inform landowners and non-governmental organizations like DUC and ALUS as to how perennial buffers can be better implemented to best protect wetland health. Fully surrounding PPR wetlands with perennial buffers could be an effective management strategy for reducing pesticide and nutrient contamination and enhancing biodiversity and ecosystem services of PPR wetlands.

Chapter 4: Synthesis and Recommendations

4.1 Synthesis

This research investigated multiple wetland responses to agricultural stressors alongside vegetative mitigation measures. Wetlands of the PPR are frequently impacted by both physical and chemical agricultural stressors, and here I aimed to characterize the associated ecotoxicological effects with particular attention on identifying key abiotic and biotic indicators of disturbance and pollution. Furthermore, perennial vegetation buffers, a tool that has been promoted for protecting agriculturally impacted water bodies, may offer a solution for protecting impacted PPR wetlands. The results discussed here could help inform future systematic biomonitoring of PPR wetland health and environmentally responsible management of these wetland resources in an agriculturally intensive region.

In Chapter 2, I assessed differences in wetland health associated with cropping on PPR wetlands, including physical disturbance of wetland vegetation, chemical disturbance from pesticide and nutrient contamination, and other water quality parameters. I did not find conclusive evidence that natural wetland vegetation and its disturbance from agriculture were directly influencing pesticide or nutrient contamination profiles of wetlands. However, vegetation zone width remaining around study wetlands was significantly associated with differences in water quality assessed in a partial RDA, suggesting that encroachment of cropland into natural wetland vegetation zones could impair water quality.

Vegetation disturbance in the form of zone width and loss of vegetative cover was associated with significant differences in the aquatic invertebrate community. Wetlands with wider, less disturbed vegetation zones had higher macroinvertebrate richness and greater abundance of Odonata, a group of beneficial insects. Aspects of water quality, including cyanobacteria blooms, nutrient levels, and pesticide contamination were also found to be significantly related to aquatic invertebrate community composition. Cyanobacteria blooms and higher total nitrogen were associated with lower diversity and abundance of multiple invertebrate taxa. Greater predicted pesticide toxicity of water was associated with lower insect abundance and greater relative snail abundance, as well as a shift in proportions of functional feeding groups. Aquatic invertebrate communities are considered excellent bioindicators and are sensitive to changes in aquatic health. These findings demonstrate the importance of water quality in shaping aquatic invertebrate communities of prairie wetlands and the potential threat particularly from excess nutrient runoff from agricultural fields to wetland health. While not

measured in this study, the observed pesticide and nutrient associated shifts in taxa abundance and community dynamics could have implications for ecosystem function.

In Chapter 3, I explored water quality and ecological responses to perennial vegetation plantings implemented by farmers and conservation NGOs, DUC and ALUS. Wetlands embedded in cropland containing both natural and forage planted buffers were compared to those that had been surrounded by crop, which often displaced natural wetland vegetation. I explored the efficacy of these buffers in mitigating some of the agricultural disturbances explored in Chapter 2 (pesticide and nutrient contamination and effects on aquatic invertebrate communities). While the presence of perennial buffers was not directly associated with reductions in pesticide runoff, wetlands that were completely surrounded (protected) by perennial plantings and/or other natural vegetation area such as field margin were found to have significantly lower concentrations of pesticides and nutrients as well as lower PTIs. This suggests that there is a clear benefit to maintaining intact wetland margins and that in order to be effective at improving aquatic ecosystem health, these need to be completely encircling the wetland perimeter. Perennial vegetation in the form of forage plantings and protection from other natural vegetation were associated with greater abundance of macroinvertebrates, zooplankton, and insects. These findings suggest that perennial vegetation plantings can increase invertebrate abundance, potentially increasing secondary invertebrate productivity and ecosystem services provided by PPR wetlands.

Although the presence of vegetative buffers can improve wetland ecosystem health, one caution was that even those wetlands that were fully surrounded by perennial vegetation plantings and/or natural vegetation were still contaminated with quantifiable concentrations of pesticides and therefore still vulnerable to contamination. High rates of pesticide contamination in PPR wetlands have been previously reported (Main et al., 2014). This is consistent with spatial modeling of pesticide application and environmental data which predicted that Saskatchewan wetlands are particularly vulnerable to frequent pesticide contamination (Malaj et al., 2020). The results of the current study are consistent with these previous findings, with neonicotinoid insecticides quantified in the majority the 60 wetlands sampled in either 2018 or 2019.

4.2 Recommendations for Aquatic Invertebrate Biomonitoring in PPR Wetlands

Aquatic invertebrate biomonitoring is a well-established method for assessing aquatic ecosystem health (Gaufin, 1973; Lenat, 1988; Metcalfe, 1989). Much of the framework of this practice has been developed for rivers and streams, with comparatively little of this biomonitoring research occurring in wetlands. Wetlands are extremely different ecosystems to streams, being lentic, often shallow and impermanent aquatic systems (National Wetlands Working Group & Canada Committee on Ecological Land Classification, 1988). The shallow and highly fluctuating water levels of PPR wetlands result in what would be considered poor water quality for a stream, including fluctuating and often high temperatures, high salinity, and periods of low dissolved oxygen. Collectively, this creates an aquatic environment largely composed of generalist invertebrates, absent of many stream adapted taxa (Ephemeroptera and Plecoptera) (Batzer et al., 1999). Consequently, the aquatic invertebrate biomonitoring endpoints that are useful in monitoring stream health may not be equivalent indicators for monitoring wetland health.

In traditional aquatic invertebrate biomonitoring schemes, several indicators are commonly used to measure aquatic ecosystem health – richness, diversity, evenness, dominance, and the presence of selected taxa (e.g., Ephemeroptera, Plecoptera, and Trichoptera) (Gaufin, 1973; Lenat, 1988; Mandaville, 2002; Metcalfe, 1989). Due to the limited number of studies that have previously examined PPR wetland health through an aquatic invertebrate biomonitoring framework (Cavallaro et al., 2019; Schepker et al., 2020), this study examined a suite of biomonitoring endpoints to determine how wetland aquatic invertebrate communities were influenced by different stressors. Multivariate techniques were also used to assess differences in the wetland invertebrate community. Traditional biotic indices such as Shannon's Diversity, Shannon's Evenness, and Berger-Parker Dominance were associated with multiple nutrient parameters in wetlands. This suggests that these well-established metrics could be useful for monitoring PPR wetland health in response to contamination with excess nutrients.

In contrast to their association with nutrient parameters, these biotic indices were *not* related to predicted pesticide toxicity measured as "chronic PTI" in water samples. Pesticide contamination detected in study wetlands sampled over three monthly intervals in this study, was widespread and at levels predicted to cause effects to aquatic life based on calculated PTIs (Nowell et al., 2014). However, PTI was not significantly associated with changes in richness,

Shannon's Diversity, Shannon's Evenness, and Berger-Parker Dominance, or Hilsenhoff Biotic Index. Conversely, PTI *was* significantly associated with significantly lower in insect abundance, higher relative snail abundance, and a shift in functional feeding groups. These findings suggest that traditional biotic indices may be inadequate in detecting changes in aquatic invertebrate communities of PPR wetlands as a result of pesticide contamination at the levels reported in this study. Based on the current study, the most useful endpoints for monitoring the effects of pesticides in PPR wetlands might be relative insect abundance and relative snail abundance. Previous studies have focused on the emergent insect taxa (Cavallaro et al., 2018, 2019). However, here I highlight the importance of monitoring snail abundance in wetlands which requires below water sampling (e.g., CABIN wetlands sampling) to effectively evaluate the broader invertebrate community.

This study could serve as a framework for future PPR wetland biomonitoring. The CABIN protocol for wetlands was introduced in 2018 (Environment and Climate Change Canada, 2018) and has not been widely validated for its utility to monitor for agricultural threats. Wetlands in the PPR are poorly characterized and often under threat of being drained or degraded due to agricultural activities. These threats to PPR wetlands make it particularly important for researchers to adopt relevant tools for monitoring the health of these threatened ecosystems.

4.3 Recommendations for Implementing Perennial Vegetative Buffers

Vegetation buffers have been promoted and researched for their ability to mitigate pesticide and nutrient runoff into water bodies (Prosser et al., 2020). Although the efficacy of implementing vegetation buffers had yet to be studied in the context of PPR wetlands, previous work has examined the ability of natural wetland vegetation in reducing pesticide contamination of wetlands (Main et al., 2015). Authors of this previous study found that wetlands with certain natural vegetation communities in their outer zones were less likely to be contaminated with neonicotinoids. A separate study found that concentrations of neonicotinoids were lower in wetlands with ≥ 50 m of natural vegetation between the wetland and cropland (Schepker et al., 2020). Both studies provide evidence that natural vegetation around wetlands can act as a buffer, reducing pesticide contamination.

Building on this previous work, the current study investigated whether natural wetland vegetation or implemented perennial vegetation buffers were effective at mitigating pesticide and nutrient contamination of PPR wetlands. Natural wetland vegetation and its disturbance from agriculture were not found to have direct effects on pesticide or nutrient concentrations in study wetlands; however wetlands surrounded by perennial vegetation buffers and/or other natural vegetation were found to have significantly lower pesticide and nutrient concentrations. Our findings suggest that perennial vegetation plantings could serve as a useful tool in reducing pesticide and nutrient contamination of wetlands and enhancing aquatic biodiversity. Considering the results of this study, it is advisable that perennial vegetation plantings be implemented in a configuration that fully surrounds a wetland if the effects of pesticide and nutrient runoff mitigation are desired.

4.4 Implications for PPR Wetland Conservation

The majority of the wetlands of the PPR have likely already been lost due to drainage (Doherty et al., 2018). Many of those that still exist are themselves at risk of being drained or degraded due to agricultural activities (Bartzen et al., 2010; Johnston, 2013). Understanding how PPR wetlands are being impacted by agriculture and what this means for wetland health can help land managers and conservation organizations in prioritizing actions for protecting these valuable ecosystems. Consistent with Bartzen et al. (2010) and Main et al. (2015), this study found widespread vegetation disturbance and pesticide contamination from agriculture in all of the sampled study wetlands. This study also found that pesticide contamination was significantly associated with differences in aquatic invertebrate communities, an indicator of ecosystem health. Pesticide contamination in particular was associated with shifts in aquatic invertebrate community composition and reduced insect abundance. Previous work has linked neonicotinoid contamination detected in wetlands to declines in insect emergence (Cavallaro et al., 2019) and reduced aquatic invertebrate biomass (Schepker et al., 2020), both of which could be explained by the pesticide associated reduction in aquatic insect abundance observed in the current study. Other parameters in addition to pesticide contamination, including concentration of nutrients, the occurrence of cyanobacteria blooms, and the degree of vegetation disturbance were found to collectively modify aquatic invertebrate community composition of wetlands.

Shifts in wetland aquatic invertebrate communities, particularly reduced aquatic insect abundance, could impact ecosystem function and reduce the productivity of PPR wetlands which are relied upon by many migratory birds for food. Aerial insectivores utilize PPR wetlands for their production of emergent aquatic insect prey and preferentially forage around them (Elgin et al., 2020; Michelson et al., 2018). Emergent aquatic insects contain high levels of fatty acids, making them a more nutritious prey item than terrestrial insects for foraging birds (Twining et al., 2019). Consequently, loss of PPR wetlands or the ecosystem services they provide could have broader implications for the health of surrounding wildlife and ecosystems.

4.4 Study Limitations and Future Work

While this study begins to examine the complex interactions between PPR wetland health and the many stressors that impact it, there are still many unanswered questions. The suite of parameters measured in each study wetland limited this research to a small sample size relative to the high degree of water quality and ecological variation found in PPR wetlands. Pesticide contamination of wetland water was analyzed in grab samples collected at up to three time points throughout the summer. Although grab sampling is widely used, it often lacks temporal resolution and has been demonstrated by researchers in one study to underestimate pesticide concentrations throughout the entire sampling period by 50% and maximum concentrations by 1 to 3 orders of magnitude (Xing et al., 2013). Although neonicotinoids can persist in soil for over a year (Schaafsma et al., 2016), they can degrade through UV irradiation once in water where they can be exposed to sunlight (Acero et al., 2019). It is therefore likely that grab samples taken for pesticide analysis in this study did not capture peak pesticide concentrations and are not completely reflective of the level of pesticide contamination that actually occurred in wetlands throughout the summer. As a result, predicted toxicity of pesticide mixtures summarized as PTIs are likely an underestimation of the pesticide toxicity that occurred in study wetlands. Increasing wetland sample size and frequency of pesticide sampling would help characterize pesticide contamination of wetlands more accurately, thus improving our understanding of the risks these contaminants pose to aquatic life.

In this study I was able to determine that wetlands completely surrounded by perennial buffer and/or other natural vegetation had lower pesticide and nutrient contamination. However, I was unable to determine what widths or configurations of perennial vegetation plantings confer

pesticide reductions. A study that experimentally implements perennial vegetation plantings around PPR wetlands would have more control over the design and interpretation. Researchers could likely then produce clearer results, more directly understanding how perennial buffer implementation and different configurations of these buffers mitigate contaminated runoff. This could give producers and land managers clear, scientifically informed guidance for implementing perennial buffers for favorable environmental outcomes.

Another important area that requires more research is in improved monitoring of wetland health. This study identified a number of aquatic invertebrate endpoints as indicators of particular agricultural stressors. Building on this research, a future study could develop a macroinvertebrate multi-metric index (MMI) which incorporates multiple macroinvertebrate endpoints specific for wetlands to create an integrative tool which can be used to gauge the health of these unique aquatic systems (Shull et al., 2018; Silva et al., 2017; Theodoropoulos et al., 2020). An MMI designed specifically for wetlands impacted by agricultural stressors could help future researchers better evaluate what wetlands are most at risk and what remediation efforts, such as perennial buffers, are most effective in protecting PPR wetland health.

Finally, this study provided important information on the application of the CABIN protocol and the use of invertebrate biomonitoring indices that are sensitive to differences in agrochemical stressors and physical disturbances. This study provides a template to conduct widespread biomonitoring across the region and ideally over time to detect changes following implementation of environmental best management practices and changes in agrochemical use and products. For example, following special reviews by the Pest Management Regulatory Agency (PMRA), a decision on imidacloprid (Health Canada Pest Management Regulatory Agency, 2020), thiamethoxam and clothianidin (Health Canada Pest Management Regulatory Agency, 2021b, 2021a) have recently been given regulatory approval for continued use. The PMRA included conditions that suggest spray buffer zones and increased setbacks from waterways and addition of perennial buffers to protect aquatic ecosystem health, notably near wetlands. The work conducted here could be useful to monitoring efforts aimed at assessing changes over the next few years as shifts in farming practices are implemented.

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Appendix

Appendix A. LOQs of pesticides analyzed by HPLC-MS-MS by the National Hydrology Research Center, Environment and Climate Change Canada in Saskatoon, SK and Trace Residue Analysis and Immunochemistry, Agriculture and Agri-Food Canada in Lethbridge, AB in 2018 and 2019.

Compound	LOQ (µg/L)
diclorvos	0.054
allidochlor	0.026
etradiazole	0.025
chlormephos	0.025
propham	0.025
clopyralid	0.026
dicamba	0.025
mecoprop	0.025
MCPA	0.025
cycloate	0.026
dichlorprop	0.025
bromoxynil	0.025
ethalfluralin	0.125
benfluralin	0.025
phorate	0.026
quintozene	0.053
prometon	0.026
diazinon	0.025
tri-allate	0.025
clomazone	0.026
etrimphos	0.071
atrazine	0.025
simazine	0.075
pirimicarb	0.026
dichlofenthion	0.025
propyzamide	0.025
dimethoate	0.255
aldrin	0.026
fenchlorphos	0.026
desmetryn	0.026
terbutryn	0.025
b-BHC	0.025
chlorpyrifos	0.025
chlorthal-dimethyl	0.025
d-BHC	0.153
fenthion	0.025

trans heptachlor epoxide	0.082
op-DDE	0.055
picloram	0.072
bromophos-ethyl	0.026
diphenamid	0.025
cis-chlordane	0.025
flumetralin	0.026
dieldrin	0.026
op-DDD	0.025
tetrasul	0.026
ethion	0.054
imazethapyr	0.108
diclofop-acid	0.025
sulprophos	0.025
mirex	0.025
methoxychlor	0.025
tetradifon	0.025
fenoxaprop	0.052
cis-permethrin	0.025
trans-permethrin	0.025
EPTC	0.025
butylate	0.026
dichlobenil	0.025
nitrapyrin	0.025
chloroneb	0.025
2,4-D	0.025
trifluralin	0.025
sulfotep	0.026
alpha-BHC	0.025
terbufos	0.053
fonofos	0.026
dioxathion	0.154
lindane	0.025
heptachlor	0.026
2,4-DB	0.025
chlorpyrifos-methyl	0.053
dimethachlor	0.025
alachlor	0.025
pirimphos-methyl	0.025
pirimphos-ethyl	0.052
metolachlor	0.025
terbacil	0.107
quinclorac	0.026

butralin	0.025
bromacil	0.026
ethofumesate	0.025
isofenphos	0.051
chlorthiamide	0.025
alpha-endosulphan	0.085
t-chlordane	0.026
butachlor	0.082
pp-DDE	0.025
procymidon	0.025
endrin	0.025
op-DDT	0.025
flamprop-methyl	0.025
bupirimate	0.025
flamprop-isopropyl	0.025
pp-DDD	0.026
pp-DDT	0.062
benalaxyl	0.025
bifenthrin	0.026
benzoylprop-ethyl	0.026
bromopropylate	0.050
triclopyr	0.025
fluroxypyr	0.026
bentazon	0.025
2,4-diclorophenol	0.152
clodinafop-propargyl	0.152
propiconazole	0.071
Imazamethabenz	0.050
Iprodione	0.025
Chlorothalonil	0.052
Prothioconazole-desthio	0.025
Boscalid	0.025
Tebuconazole	0.051
Difenoconazole	0.102
Carbaryl	0.051
Malathion	0.025
Trifloxystrobin	0.026
Pyraclostrobin	0.156
MonoLinuron	0.610
Vinclozolin	0.026
Azoxystrobin	0.113
Hexazinone	0.051
Quizalofop-ethyl	0.025

Triticonazole	0.154
Cyhalothrin lambda	0.026
Carfentrazone-ethyl	0.025
Fluazifop-p-butyl	0.025
Fludioxonil	0.025
Metalaxyl	0.025
Pendimethalin	0.025
Naled	0.151
Deltamethrine	0.025
Prometryn	0.026
Captan	0.059
MCPB-methyl	0.025
Carbofuran	0.025
Napropamide	0.025
Piperonyl butoxide	0.025
Methoprene	0.071
Oxyfluorfen	0.123
Azinphos-methyl	0.511
Folpet	0.212
Propoxur	0.025
Bifenazate	0.131
Cyfluthrin	0.073
Cypermethrin-beta	0.071
Cypermethrin-zeta	0.072
Cyprodinil	0.025
Famoxadone	0.154
Fenamidone	0.026
Flumioxazin	0.073
Ipconazole	0.025
Metconazole	0.152
Myclobutanil	0.025
Picoxystrobin	0.025
Propetamphos	0.026
Pyridaben	0.025
Pyrimethanil	0.053
Spiromesifen	0.025
Sulfentrazone	0.504
Tetramethrin I	0.025
Zoxamide	0.502
MCPA-EHE	0.026
Dinotefuran	0.015
Nitenpyram	0.003
Thiamethoxam	0.002

Clothianidin	0.010
Imidacloprid	0.005
Acetamiprid	0.010
Thiacloprid	0.010
Flonicamid	0.140
Flupyradifurone	0.010
Sulfoxaflor	0.010
Cyantraniliprole	0.020
Chlorantraniliprole	0.020

Appendix B. Average recoveries of high and low pesticide concentration spiked samples analyzed by HPLC-MS/MS (n = 9 trials). Percent recoveries outside 70-120% are highlighted in blue.

Compound	High Spike in MilliQ	High Spike 100%	Percent	Low Spike in Milliq	Low Spike 100%	Percent
diclorvos	55.16	74.59	74.0	11.77	14.70	80.0
allidochlor	22.97	26.76	85.8	4.18	5.40	77.4
etradiazole	21.88	26.32	83.1	4.68	5.40	86.7
chlormephos	19.69	24.78	79.5	4.27	4.97	85.9
propham	20.57	26.45	77.8	4.56	5.26	86.7
clopyralid	9.91	18.82	52.7	1.91	4.02	47.7
dicamba	20.45	25.05	81.6	4.34	5.04	86.1
mecoprop	19.87	19.20	103.5	2.96	3.34	88.4
MCPA	20.27	25.46	79.6	4.65	5.19	89.6
cycloate	20.60	25.44	81.0	4.41	5.08	86.8
dichlorprop	18.91	18.47	102.4	2.68	3.00	89.3
bromoxynil	19.24	18.51	103.9	3.56	3.80	93.7
ethalfluralin	90.86	115.20	78.9	18.69	21.98	85.0
benfluralin	16.03	21.86	73.3	3.29	3.98	82.8
phorate	17.01	23.17	73.4	3.72	4.59	81.1
quintozene	33.96	46.54	73.0	7.88	9.07	86.9
prometon	13.49	36.18	37.3	2.97	4.47	66.6
diazinon	19.39	21.02	92.3	3.54	3.83	92.3
tri-allate	19.50	24.75	78.8	4.24	4.74	89.3
clomazone	21.27	26.56	80.1	4.49	5.18	86.7
etrimphos	51.17	64.39	79.5	10.50	11.93	88.0
atrazine	19.30	19.36	99.7	3.21	3.60	89.1
simazine	53.22	51.78	102.8	9.10	10.36	87.8
pirimicarb	14.57	23.25	62.7	5.77	4.49	128.7
dichlofenthion	19.23	24.65	78.0	4.11	4.84	85.0
propyzamide	19.42	24.06	80.7	4.02	4.67	85.9

dimethoate	219.24	215.99	101.5	39.17	42.01	93.2
aldrin	16.35	26.05	62.8	3.62	5.39	67.1
fenchlorphos	17.78	23.07	77.1	3.83	4.55	84.3
desmetryn	17.17	39.41	43.6	3.43	6.11	56.2
terbutryn	22.37	20.95	106.7	4.18	4.08	102.3
b-BHC	19.51	24.44	79.8	4.15	4.83	86.0
chlorpyrifos	19.16	24.34	78.7	4.81	4.80	100.2
chlorthal-dimethyl	20.25	25.07	80.8	4.31	5.01	85.9
d-BHC	112.88	142.35	79.3	23.51	27.68	84.9
fenthion	17.39	22.66	76.7	3.74	4.33	86.3
trans heptachlor epoxide	60.71	79.18	76.7	13.43	16.02	83.8
op-DDE	39.37	54.15	72.7	8.23	10.73	76.7
picloram	29.45	48.78	60.4	3.81	8.10	47.0
bromophos-ethyl	18.37	23.90	76.9	3.89	4.83	80.6
diphenamid	21.01	25.28	83.1	4.40	5.03	87.4
cis-chlordane	18.24	24.60	74.1	3.82	4.71	81.1
flumetralin	18.06	21.85	82.6	3.81	4.43	86.0
dieldrin	20.45	25.88	79.0	5.00	5.75	87.0
op-DDD	18.85	24.64	76.5	3.99	4.88	81.9
tetrasul	18.61	25.02	74.4	4.02	5.03	79.9
ethion	46.50	52.55	88.5	9.19	10.01	91.8
imazethapyr	117.04	122.65	95.4	18.22	21.58	84.4
diclofop-acid	19.16	18.02	106.4	2.80	2.97	94.2
sulprophos	16.35	21.40	76.4	3.33	4.14	80.4
mirex	16.35	22.52	72.6	3.16	4.38	72.3
methoxychlor	22.45	25.12	89.4	4.68	4.84	96.6
tetradifon	18.69	23.32	80.1	4.20	4.96	84.6
fenoxaprop	39.85	47.34	84.2	5.51	7.36	74.9
cis-permethrin	23.22	26.12	88.9	5.11	5.36	95.2
trans-permethrin	23.27	26.02	89.4	4.52	5.32	85.0
EPTC	20.79	25.19	82.5	4.41	5.28	83.5
butylate	19.18	25.19	76.2	4.21	5.08	82.9
dichlobenil	21.75	26.69	81.5	4.43	5.26	84.2
nitrapyrin	19.01	24.01	79.2	4.52	4.70	96.3
chloroneb	20.20	25.24	80.0	4.35	5.03	86.4
2,4-D	18.46	19.17	96.3	2.18	2.32	94.2
trifluralin	18.43	23.73	77.7	3.89	4.68	83.1
sulfotep	19.95	24.78	80.5	4.35	5.06	86.1
alpha-BHC	19.52	24.33	80.2	4.15	4.79	86.5
terbufos	31.06	45.07	68.9	6.55	8.64	75.8
fonofos	19.99	25.00	80.0	4.34	5.10	85.0
dioxathion	153.63	169.14	90.8	33.44	34.61	96.6

lindane	19.63	24.27	80.9	4.17	4.80	86.8
heptachlor	16.34	24.05	67.9	3.58	4.68	76.5
2,4-DB	18.97	18.83	100.7	3.24	3.86	83.9
chlorpyrifos-methyl	37.91	48.76	77.7	8.12	9.51	85.4
dimethachlor	21.49	25.75	83.5	4.76	5.01	94.9
alachlor	20.34	24.43	83.2	4.45	4.93	90.3
pirimphos-methyl	18.74	19.26	97.3	3.36	3.25	103.4
pirimphos-ethyl	37.67	40.20	93.7	6.85	7.38	92.8
metolachlor	20.11	24.25	82.9	4.34	4.79	90.6
terbacil	46.33	46.23	100.2	8.75	10.97	79.7
quinclorac	18.90	18.45	102.4	2.40	2.66	90.4
butralin	19.44	22.42	86.7	3.90	4.52	86.3
bromacil	6.32	9.06	69.8	1.11	1.73	63.9
ethofumesate	20.68	25.74	80.3	4.53	5.02	90.2
isofenphos	37.47	46.44	80.7	7.75	8.94	86.7
chlorthiamide	21.38	26.31	81.3	4.43	5.17	85.8
alpha-endosulphan	64.28	82.26	78.1	13.77	16.38	84.0
t-chlordane	18.14	24.46	74.1	3.93	4.87	80.7
butachlor	61.08	75.15	81.3	12.59	14.21	88.6
pp-DDE	18.32	24.78	73.9	3.85	4.97	77.5
procymidon	20.19	24.99	80.8	4.32	5.03	85.8
endrin	19.92	24.82	80.3	4.36	5.24	83.2
op-DDT	16.72	22.95	72.8	3.49	4.29	81.2
flamprop-methyl	21.50	25.59	84.0	4.57	5.09	89.9
bupirimate	20.08	20.08	100.0	3.55	3.90	91.2
flamprop-isopropyl	21.55	25.64	84.0	4.74	5.27	89.8
pp-DDD	16.83	22.93	73.4	3.41	4.44	76.7
pp-DDT	49.27	60.54	81.4	9.77	11.54	84.6
benalaxyl	20.90	24.73	84.5	4.37	4.79	91.2
bifenthrin	18.64	29.10	64.1	7.89	8.66	91.1
benzoylprop-ethyl	20.83	24.24	85.9	4.15	4.60	90.2
bromopropylate	38.45	47.01	81.8	8.20	9.38	87.5
triclopyr	18.86	18.44	102.3	2.56	2.79	91.5
fluroxypyr	25.38	24.42	103.9	3.30	4.07	81.0
bentazon	21.40	20.57	104.1	3.70	3.96	93.6
2,4-diclorophenol	33.69	82.78	40.7	10.06	15.79	63.7
clodinafop-propargyl	114.96	125.82	91.4	19.30	19.81	97.4
propiconazole	55.72	55.51	100.4	9.83	10.66	92.2
Imazamethabenz	38.10	39.13	97.4	8.08	10.44	77.4
Iprodione	20.72	24.20	85.6	4.01	4.73	84.8
Chlorothalonil	13.61	20.55	66.2	0.15	0.24	62.9

Prothioconazole-desthio	20.84	21.09	98.8	3.74	3.99	93.8
Boscalid	19.24	21.97	87.6	3.86	4.18	92.3
Tebuconazole	48.78	45.39	107.5	8.86	8.85	100.1
Difenoconazole	110.62	91.57	120.8	18.87	16.60	113.7
Carbaryl	41.52	47.57	87.3	8.04	9.81	81.9
Malathion	18.12	21.99	82.4	3.94	4.64	84.9
Trifloxystrobin	18.82	29.01	64.9	3.73	4.19	89.1
Pyraclostrobin	126.53	144.95	87.3	25.42	27.17	93.5
MonoLinuron	372.11	549.06	67.8	81.64	106.57	76.6
Vinclozolin	19.32	25.82	74.8	4.35	5.10	85.3
Azoxystrobin	136.09	96.81	140.6	24.53	17.31	141.7
Hexazinone	63.35	54.52	116.2	11.78	11.89	99.0
Quizalofop-ethyl	30.08	31.10	96.7	5.81	5.55	104.6
Triticonazole	173.32	156.05	111.1	29.50	30.88	95.5
Cyhalothrin lambda	16.67	20.58	81.0	3.43	3.96	86.6
Carfentrazone-ethyl	23.82	26.43	90.1	4.92	5.21	94.5
Fluazifop-p-butyl	19.54	23.59	82.8	4.05	4.56	88.8
Fludioxonil	19.64	23.22	84.6	3.91	4.32	90.4
Metalaxyl	22.30	25.92	86.0	4.67	5.06	92.4
Pendimethalin	19.99	22.88	87.3	3.98	4.43	90.0
Naled	97.17	113.20	85.8	17.53	18.29	95.8
Deltamethrine	22.54	25.51	88.4	4.88	5.36	91.0
Prometryn	21.72	19.24	112.9	4.19	3.85	109.0
Captan	25.11	58.23	43.1	8.11	11.32	71.7
MCPB-methyl	19.60	24.57	79.8	4.09	4.86	84.1
Carbofuran	22.07	24.57	89.8	4.32	4.91	88.0
Napropamide	21.84	24.12	90.6	4.78	5.03	95.0
Piperonyl butoxide	28.34	24.20	117.1	5.36	4.78	112.0
Methoprene	47.34	59.75	79.2	10.32	11.40	90.5
Oxyfluorfen	99.74	115.00	86.7	18.97	20.19	94.0
Azinphos-methyl	362.34	446.14	81.2	78.03	82.11	95.0
Folpet	83.48	228.13	36.6	22.85	42.21	54.1
Propoxur	21.50	25.42	84.6	4.41	4.93	89.5
Bifenazate	117.22	151.75	77.2	27.66	33.30	83.1
Cyfluthrin	65.23	76.37	85.4	15.01	16.75	89.6
Cypermethrin-beta	50.38	72.99	69.0	15.42	17.61	87.6
Cypermethrin-zeta	76.63	68.44	112.0	28.25	21.32	132.5
Cyprodinil	19.29	19.35	99.7	3.41	3.82	89.2
Famoxadone	121.65	124.95	97.4	27.13	27.18	99.8
Fenamidone	8.94	7.54	118.7	1.64	1.44	113.9
Flumioxazin	55.31	59.67	92.7	10.31	11.50	89.7
Ipconazole	19.26	19.33	99.6	3.72	4.18	89.2

Metconazole	117.44	116.63	100.7	20.10	21.43	93.8
Myclobutanil	25.11	23.24	108.0	4.47	4.53	98.7
Picoxystrobin	20.79	24.98	83.2	4.43	5.02	88.2
Propetamphos	20.40	24.46	83.4	4.39	5.04	87.1
Pyridaben	22.58	25.10	89.9	4.61	4.80	96.0
Pyrimethanil	39.73	35.22	112.8	7.49	7.91	94.7
Spiromesifen	22.20	19.13	116.1	4.66	4.97	93.8
Sulfentrazone	829.12	1532.93	54.1	301.20	398.17	75.6
Tetramethrin I	15.75	24.43	64.5	4.12	4.60	89.6
Zoxamide	323.40	416.09	77.7	67.51	81.83	82.5
MCPA-EHE	19.92	24.89	80.0	10.52	4.92	214.0

Appendix C. Toxicity values used in calculations of acute and chronic PTIs. Values area either an HC_5 obtained from an acute or chronic SSD or (in cases when fewer than 7 values were available for SSDs) a minimum toxicity value (minimum) representing the most sensitive published toxicity value for an aquatic species.

Pesticide	Chronic Value (µg/L)	Chronic Value Type	Acute Value (µg/L)	Acute Value Type
imidacloprid	0.04	HC_5	0.19	HC_5
thiamethoxam	0.026	HC_5	9	HC_5
clothianidin	0.0015	HC_5	1.5	HC_5
acetamiprid	0.04	minimum	1.9	HC_5
chlorantraniliprole	4.05	minimum	4.62	HC_5
cyantraniliprole	10.25	minimum	14.1	minimum
flonicamid	3100	minimum	97900	minimum
2,4-D	9.211297341	HC_5	3350.647453	HC_5
bifenthrin	0.000509764	HC_5	0.017905165	HC_5
bromoxynil	2270	minimum	1900	minimum
clopyralid	6900	minimum	4000	minimum
dicamba	15.93825881	HC_5	2605.76198	HC_5
fluroxypyr	3047.49799	minimum	14300	minimum
imazamethabenz	NA*	minimum	89100	minimum
imazethapyr	8.1	minimum	110000	minimum
iprodione	22.75352906	HC_5	480	minimum
MCPA	36.03846997	HC_5	3712.893193	HC_5
metalaxyl	542.9237237	HC_5	1956.987291	HC_5
picoxystrobin	8	minimum	22	minimum
prothioconazole-desthio	3.4	minimum	81	minimum
quinclorac	19.4395435	HC_5	500	minimum
tebuconazole	10.77409469	HC_5	701.2645488	HC_5

triallate	10	minimum	57	minimum
trifloxystrobin	0.2	minimum	1.7	minimum

*acute values used in place of chronic values if chronic value not available

Appendix D. Correlation matrix of Pearson's r correlation coefficients of water quality variables measured in 32 Saskatchewan wetlands in the growing season of 2018. Values of 0.70 or greater are in bold.

	Ammonia	Bicarbonate	Ca	Carbonate	Cl	Conductivit	F	Fe	Mg	Mn
Chronic PTI	-0.16	-0.21	-0.11	0.02	-0.27	-0.23	0.15	-0.11	-0.19	-0.10
TOC	0.17	0.63	0.49	-0.15	-0.03	0.30	0.33	0.18	0.21	**0.70**
TN	**0.76**	**0.74**	0.31	-0.39	0.07	0.37	0.24	0.37	0.30	0.42
Hardness	0.18	0.25	**0.70**	-0.07	0.16	**0.99**	0.05	0.14	**0.99**	0.01
TDS	0.23	0.33	**0.72**	-0.13	0.17	**0.99**	0.07	0.19	**0.98**	0.05
Total Alkalinity	0.34	**0.87**	0.29	-0.14	0.04	0.29	0.25	0.27	0.24	0.26
Sulfate	0.11	0.04	0.65	-0.03	0.14	**0.96**	-0.02	0.10	**0.97**	-0.05
Na	0.16	0.08	0.44	0.19	0.31	**0.91**	-0.11	0.15	**0.94**	-0.17
K	0.06	0.27	0.32	-0.35	0.10	0.01	0.11	0.08	-0.11	0.23
TP	0.35	0.64	0.37	-0.52	0.15	0.04	0.36	0.30	-0.11	0.46
Ortho Phos.	0.39	0.59	0.34	-0.51	0.15	0.01	0.35	0.11	-0.13	0.40
pH	-0.38	-0.67	-0.43	**0.94**	0.21	-0.20	-0.27	-0.30	-0.08	-0.67
Phenol Alkalinity	-0.39	-0.61	-0.32	1.00	0.12	-0.12	-0.21	-0.30	-0.02	-0.47
NPOC	0.17	0.60	0.47	-0.06	-0.03	0.35	0.33	0.01	0.29	0.59
Mn	0.05	0.44	0.42	-0.47	-0.30	0.03	0.31	0.10	-0.07	1.00
Mg	0.21	0.20	0.59	-0.02	0.18	**0.98**	-0.02	0.13	1.00	-0.07
Fe	0.30	0.37	0.13	-0.31	0.03	0.19	-0.28	1.00	0.13	0.10
F	-0.01	0.31	0.33	-0.21	-0.14	0.04	1.00	-0.28	-0.02	0.31
Conductivity	0.24	0.29	**0.71**	-0.12	0.24	1.00	0.04	0.19	**0.98**	0.03
Cl	0.11	-0.03	-0.02	0.12	1.00	0.24	-0.14	0.03	0.18	-0.30
Carbonate	-0.39	-0.61	-0.32	1.00	0.12	-0.12	-0.21	-0.31	-0.02	-0.47
Ca	-0.01	0.39	1.00	-0.32	-0.02	**0.71**	0.33	0.13	0.59	0.42
Bicarbonate	0.47	1.00	0.39	-0.61	-0.03	0.29	0.31	0.37	0.20	0.44
Ammonia	1.00	0.47	-0.01	-0.39	0.11	0.24	-0.01	0.30	0.21	0.05

NPO	Phenol	pH	Ortho Phos.	TP	K	Na	Sulfate	Total	TDS	Hardness	TN	TOC
-0.27	0.02	0.08	-0.18	-0.20	-0.05	-0.26	-0.15	-0.24	-0.22	-0.19	-0.26	-0.26
0.96	-0.15	-0.35	0.43	0.53	0.33	0.17	0.13	0.69	0.33	0.28	**0.73**	1.00
0.68	-0.38	-0.48	0.51	0.59	0.26	0.24	0.18	0.68	0.39	0.32	1.00	**0.73**
0.34	-0.07	-0.15	-0.05	-0.03	-0.04	**0.91**	**0.97**	0.26	**0.99**	1.00	0.32	0.28
0.38	-0.14	-0.22	0.02	0.05	0.02	**0.90**	**0.95**	0.33	1.00	**0.99**	0.39	0.33
0.71	-0.14	-0.26	0.42	0.48	0.12	0.22	0.04	1.00	0.33	0.26	0.68	0.69
0.19	-0.03	-0.09	-0.16	-0.14	-0.06	**0.90**	1.00	0.04	**0.95**	**0.97**	0.18	0.13
0.24	0.19	0.11	-0.18	-0.13	-0.25	1.00	**0.90**	0.22	**0.90**	**0.91**	0.24	0.17
0.30	-0.35	-0.33	0.48	0.44	1.00	-0.25	-0.06	0.12	0.02	-0.04	0.26	0.33
0.42	-0.52	-0.53	**0.95**	1.00	0.44	-0.13	-0.14	0.48	0.05	-0.03	0.59	0.53
0.40	-0.51	-0.51	1.00	**0.95**	0.48	-0.18	-0.16	0.42	0.02	-0.05	0.51	0.43
-0.27	**0.93**	1.00	-0.51	-0.53	-0.33	0.11	-0.09	-0.26	-0.22	-0.15	-0.48	-0.35
-0.06	1.00	**0.93**	-0.51	-0.52	-0.35	0.19	-0.03	-0.14	-0.14	-0.07	-0.38	-0.15
1.00	-0.06	-0.27	0.40	0.42	0.30	0.24	0.19	**0.71**	0.38	0.34	0.68	**0.96**
0.59	-0.47	-0.67	0.40	0.46	0.23	-0.17	-0.05	0.26	0.05	0.01	0.42	**0.70**
0.29	-0.02	-0.08	-0.13	-0.11	-0.11	**0.94**	**0.97**	0.24	**0.98**	**0.99**	0.30	0.21
0.01	-0.30	-0.30	0.11	0.30	0.08	0.15	0.10	0.27	0.19	0.14	0.37	0.18
0.33	-0.21	-0.27	0.35	0.36	0.11	-0.11	-0.02	0.25	0.07	0.05	0.24	0.33
0.35	-0.12	-0.20	0.01	0.04	0.01	**0.91**	**0.96**	0.29	**0.99**	**0.99**	0.37	0.30
-0.03	0.12	0.21	0.15	0.15	0.10	0.31	0.14	0.04	0.17	0.16	0.07	-0.03
-0.06	1.00	**0.94**	-0.51	-0.52	-0.35	0.19	-0.03	-0.14	-0.13	-0.07	-0.39	-0.15
0.47	-0.32	-0.43	0.34	0.37	0.32	0.44	0.65	0.29	**0.72**	**0.70**	0.31	0.49
0.60	-0.61	-0.67	0.59	0.64	0.27	0.08	0.04	**0.87**	0.33	0.25	**0.74**	0.63
0.17	-0.39	-0.38	0.39	0.35	0.06	0.16	0.11	0.34	0.23	0.18	**0.76**	0.17

																						Chronic
-0.16	-0.21	-0.11	0.02	-0.27	-0.23	0.15	-0.11	-0.19	-0.10	-0.27	0.02	0.08	-0.18	-0.20	-0.05	-0.26	-0.15	-0.24	-0.22	-0.19	-0.26	-0.26	1.00

Appendix E. Pearson's correlation coefficients of continuous fixed effects used in a partial RDA of water quality parameters measured in 32 Saskatchewan wetlands in 2018.

	Area	Vegetation Disturbance	Zone Width	Percent Crop
Area	1.00	0.21	0.32	-0.04
Vegetation Disturbance	0.21	1.00	-0.50	-0.06
Zone Width	0.32	-0.50	1.00	0.03
Percent Crop	-0.04	-0.06	0.03	1.00

Appendix F. Pearson's correlation coefficients of continuous fixed effects used in global linear mixed effect model of cube root pesticide concentrations detected in 34 Saskatchewan wetlands in 2018.

	Area	Vegetation Disturbance	Zone Width	Percent Crop
Area	1.00	0.22	0.33	-0.05
Vegetation Disturbance	0.22	1.00	-0.52	-0.07
Zone Width	0.33	-0.52	1.00	0.02
Percent Crop	-0.05	-0.07	0.02	1.00

Appendix G. Model selection results for linear mixed effects models to examine the effects of vegetation disturbance and environmental variables on pesticide concentrations measured in 34 study wetlands in the growing season of 2018. Global model includes the fixed effects of wetland area, vegetation disturbance, zone width, percent crop, wetland depth, crop type, and sampling period. Only models with ΔAICc < 5 are reported.

Response	Model Structure	[1]k	[2]AICc	[3]ΔAICc	[4]weight
Cube Root(Pesticice Concentration)	null	3	22.67	0	0.53
	sampling period	4	22.89	0.22	0.47
	global	10	77.79	55.12	

[1]k: number of estimated parameters in the model
[2]AICc: Akaike's Information Criterion corrected for small sample sizes
[3]ΔAICc: different from AICc of the best approximating model
[4]weight: AICc weight, provided for models with ΔAICc < 5

Appendix H. Model selection results for linear models to examine the effects of vegetation disturbance and environmental variables on chronic PTI measured in 32 study wetlands in the growing season of 2018. Global model includes the fixed effects of wetland area, vegetation disturbance, zone width, percent crop, wetland depth, crop type, and block. Only models with ΔAICc < 5 are reported.

Response	Model Structure	[1]k	[2]AICc	[3]ΔAICc	[4]weight
log(Chronic PTI)	null	2	106.02	0	0.23
	crop type	3	106.53	0.51	0.18
	area	3	107.48	1.46	0.11
	veg. disturbance	3	107.83	1.81	0.09
	zone width	3	108.33	2.31	0.07
	percent crop	3	108.45	2.43	0.07
	depth	3	108.46	2.44	0.07
	crop type + veg disturbance	4	109.2	3.18	0.05
	crop type + percent crop	4	109.22	3.2	0.05
	area + crop type	4	109.23	3.21	0.05
	crop type + zone width	4	109.27	3.25	0.05
	global	9	144.17	38.15	

[1]k: number of estimated parameters in the model
[2]AICc: Akaike's Information Criterion corrected for small sample sizes
[3]ΔAICc: different from AICc of the best approximating model
[4]weight: AICc weight, provided for models with ΔAICc < 5

Appendix I. Pearson's correlation coefficients of continuous fixed effects used in global linear models of aquatic invertebrate community endpoints measured in 27 Saskatchewan wetlands in 2018. Variables include non-purgeable organic carbon (NPOC), total nitrogen (TN), and total phosphorus (TP).

	log(chronic PTI)	Conductivity	pH	TP	TN	NPOC	Veg Disturbance	Percent Crop	Zone Width	Area
Area	-0.47	0.01	0.04	0.35	0.26	0.08	0.23	-0.04	0.32	1.00
Zone Width	0.13	-0.14	-0.24	0.26	-0.08	0.17	-0.50	0.03	1.00	0.32
Percent Crop	0.23	-0.42	-0.25	0.41	0.24	0.12	-0.06	1.00	0.03	-0.04
Veg Disturbance	-0.40	0.34	-0.25	0.03	0.33	0.08	1.00	-0.06	-0.50	0.23
NPOC	-0.25	0.30	-0.24	0.43	0.66	1.00	0.08	0.12	0.17	0.08
TN	-0.25	0.32	-0.42	0.59	1.00	0.66	0.33	0.24	-0.08	0.26
TP	-0.19	-0.01	-0.47	1.00	0.59	0.43	0.03	0.41	0.26	0.35
pH	-0.08	-0.11	1.00	-0.47	-0.42	-0.24	-0.25	-0.25	-0.24	0.04
Conductivity	-0.17	1.00	-0.11	-0.01	0.32	0.30	0.34	-0.42	-0.14	0.01
log(chronic PTI)	1.00	-0.17	-0.08	-0.19	-0.25	-0.25	-0.40	0.23	0.13	-0.47

Appendix J. Model selection results for linear models to examine effects of multiple stressors on aquatic invertebrate endpoints in 27 study wetlands in the growing season of 2018. Global models include the fixed effects of zone width, vegetation disturbance, percent crop, wetland depth, crop type, wetland area, occurrence of cyanobacteria blooms, conductivity, pH, NPOC, total nitrogen, total phosphorus, log chronic PTI, and block. All models with ΔAICc < 2 are reported and up to 10 models are reported with ΔAICc < 5.

Response	Model Structure	[1]k	[2]AICc	[3]ΔAICc	[4]weight
log(Macroinvertebrate Abundance)	cyanobacteria + pH	4	22.42	0	0.27
	cyanobacteria + pH + TN	5	23.91	1.49	0.13
	conductivity + cyanobacteria + pH	5	24.04	1.61	0.12
	cyanobacteria + TN	4	24.84	2.42	0.08
	cyanobacteria + pH + TN + TP	6	24.96	2.54	0.08
	cyanobacteria + pH + zone width	5	25.12	2.7	0.07
	cyanobacteria + pH + TP	5	25.21	2.79	0.07
	cyanobacteria + NPOC + pH	5	25.26	2.84	0.07
	area + cyanobacteria + pH	5	25.29	2.86	0.06
	cyanobacteria + percent crop + pH	5	25.35	2.93	0.06
	null	2	31.14	8.72	
	global	16	187.02	164.6	
log(Zooplankton Abundance)	depth + zone width	4	33.78	0	0.17
	depth	3	34.39	0.61	0.12
	depth + veg disturbance	4	34.61	0.83	0.11
	depth + TP	4	35.34	1.56	0.08
	depth + veg disturbance + TN	5	35.35	1.57	0.08
	depth + percent crop + zone width	5	35.58	1.8	0.07
	crop type + depth + TP	5	35.65	1.87	0.07
	depth + veg disturbance + TP	5	35.66	1.87	0.06
	crop type + depth + TN + zone width	6	35.68	1.89	0.06
	depth + TN + zone width	5	35.68	1.89	0.06
	crop type + depth + TN	5	35.68	1.9	0.06
	depth + percent crop	4	35.77	1.99	0.06
	null	2	39.22	5.44	
	global	16	218.15	184.37	
Richness	cyanobacteria + NPOC + TN + zone width	6	157.18	0	0.16
	NPOC + TN + zone width	5	157.59	0.41	0.13
	cyanobacteria + NPOC + percent crop + TN + zone width	7	157.72	0.53	0.13
	conductivity + cyanobacteria + NPOC + TN + zone width	7	157.81	0.63	0.12

	conductivity + NPOC + TN + zone width	6	158.04	0.86	0.11
	cyanobacteria + NPOC + TN	5	158.39	1.21	0.09
	cyanobacteria + NPOC + percent crop + TN	6	158.9	1.72	0.07
	depth + NPOC + TN + zone width	6	158.95	1.76	0.07
	NPOC + log chronic PTI + TN + zone width	6	159.06	1.88	0.06
	conductivity + NPOC + log chronic PTI + TN + zone width	7	159.31	2.12	0.06
	null	2	172.55	15.37	
	global	16	339.77	182.59	
Hilsenhoff Biotic Index	area + conductivity + cyanobacteria + veg disturbance	6	50.74	0	0.21
	cyanobacteria + depth + veg disturbance + TN + TP	7	51.64	0.9	0.14
	area + cyanobacteria + veg disturbance	5	52.42	1.68	0.09
	area + conductivity + cyanobacteria + veg disturbance + percent crop	7	52.44	1.69	0.09
	crop type + cyanobacteria + depth + veg disturbance + percent crop + TN + TP	9	52.44	1.7	0.09
	area + cyanobacteria + depth + veg disturbance + TN	7	52.73	1.98	0.08
	area + cyanobacteria + veg disturbance + TN	6	52.73	1.99	0.08
	area + conductivity + cyanobacteria + veg disturbance + TN	7	52.82	2.08	0.08
	area + cyanobacteria + depth + veg disturbance	6	52.85	2.11	0.07
	cyanobacteria + depth + veg disturbance	5	53.06	2.32	0.07
	null	2	56.47	5.73	
	global	16	224.44	173.7	
Shannon's Diversity	cyanobacteria + NPOC + TN + TP	6	24.89	0	0.18
	cyanobacteria + TN + TP	5	25.18	0.29	0.15
	cyanobacteria + depth + TN + TP	6	25.43	0.54	0.14
	cyanobacteria + NPOC + TN	5	25.47	0.59	0.13
	cyanobacteria + TN	4	25.87	0.98	0.11
	cyanobacteria + TN + zone width	5	26.57	1.68	0.08
	cyanobacteria + depth + TN	5	26.65	1.76	0.07
	cyanobacteria + TN + zone width + TP	6	27.35	2.46	0.05

	cyanobacteria + NPOC + TN + zone width	6	27.53	2.64	0.05
	conductivity + cyanobacteria + TN + TP	6	27.54	2.66	0.05
	null	2	46.06	21.17	
	global	16	215.32	190.43	
Shannon's Evenness	cyanobacteria + TN + TP	5	-40.21	0	0.3
	cyanobacteria + depth + TN + TP	6	-38.15	2.06	0.11
	cyanobacteria + NPOC + TN + TP	6	-37.86	2.35	0.09
	area + cyanobacteria + TN + TP	6	-37.75	2.46	0.09
	cyanobacteria + TN	4	-37.75	2.46	0.09
	conductivity + cyanobacteria + TN + TP	6	-37.23	2.98	0.07
	cyanobacteria + log chronic PTI + TN + TP	6	-37.1	3.11	0.06
	cyanobacteria + TN + zone width + TP	6	-37.04	3.17	0.06
	cyanobacteria + pH + TN + TP	6	-37.01	3.2	0.06
	cyanobacteria + veg disturbance + TN + TP	6	-36.89	3.32	0.06
	null	2	-18.87	21.34	
	global	16	154.46	194.67	
Berger-Parker Dominance	cyanobacteria + TN + TP	5	-26.35	0	0.3
	cyanobacteria + veg disturbance + TN + TP	6	-24.18	2.17	0.1
	area + cyanobacteria + TN + TP	6	-23.93	2.42	0.09
	cyanobacteria + NPOC + TN + TP	6	-23.92	2.43	0.09
	cyanobacteria + percent crop + TN + TP	6	-23.91	2.44	0.09
	conductivity + cyanobacteria + TN + TP	6	-23.86	2.49	0.09
	cyanobacteria + depth + TN + TP	6	-23.28	3.07	0.06
	cyanobacteria + TN + zone width + TP	6	-23.23	3.12	0.06
	cyanobacteria + TN	4	-23.22	3.13	0.06
	conductivity + cyanobacteria + percent crop + TN + TP	7	-23.17	3.18	0.06
	null	2	-4.66	21.69	
	global	16	165.22	191.57	
log(Insect Abundance)	area + cyanobacteria + pH + log chronic PTI	6	22.07	0	0.37
	area + cyanobacteria + veg disturbance + pH + log chronic PTI	7	24.87	2.8	0.09
	cyanobacteria + pH	4	25.12	3.05	0.08

	area + cyanobacteria + pH + log chronic PTI + TP	7	25.13	3.06	0.08
	cyanobacteria + pH + log chronic PTI	5	25.32	3.25	0.07
	area + conductivity + cyanobacteria + pH + log chronic PTI	7	25.62	3.56	0.06
	area + cyanobacteria + NPOC + pH + log chronic PTI	7	25.65	3.58	0.06
	area + cyanobacteria + percent crop + pH + log chronic PTI	7	25.7	3.63	0.06
	area + cyanobacteria + pH + log chronic PTI + TN	7	25.71	3.65	0.06
	area + cyanobacteria + depth + pH + log chronic PTI	7	25.75	3.68	0.06
	null	2	36.13	14.06	
	global	16	198.14	176.07	
log(Snail Abundance)	cyanobacteria + depth + veg disturbance + TN + zone width	7	63.02	0	0.17
	cyanobacteria + depth + veg disturbance + percent crop + TN + zone width	8	63.62	0.6	0.13
	cyanobacteria + depth + veg disturbance + log chronic PTI + TN + zone width	8	63.64	0.61	0.13
	cyanobacteria + depth + veg disturbance + pH + log chronic PTI + TN + zone width	9	64.01	0.99	0.1
	cyanobacteria + depth + veg disturbance + NPOC + log chronic PTI + TN + zone width	9	64.23	1.21	0.09
	block + cyanobacteria + NPOC + percent crop + TN + zone width	8	64.31	1.29	0.09
	cyanobacteria + veg disturbance + NPOC + log chronic PTI + TN + zone width	8	64.56	1.53	0.08
	cyanobacteria + veg disturbance + NPOC + TN + zone width	7	64.74	1.71	0.07
	cyanobacteria + depth + veg disturbance + NPOC + TN + zone width	8	64.77	1.75	0.07
	cyanobacteria + depth + veg disturbance + percent crop + pH + log chronic PTI + TN + zone width	10	64.89	1.86	0.07
	null	2	92.9	29.88	
	global	16	226.88	163.86	
	null	2	45.88	0	0.21

log(Coleoptera Abundance)	pH	3	46.55	0.67	0.15
	cyanobacteria	3	47.14	1.26	0.11
	zone width	3	47.41	1.53	0.1
	NPOC	3	47.48	1.61	0.09
	cyanobacteria + pH	4	47.72	1.84	0.08
	area	3	48.01	2.13	0.07
	depth	3	48.12	2.24	0.07
	conductivity	3	48.25	2.37	0.06
	TP	3	48.37	2.49	0.06
	global	16	229	183.12	
log(Diptera Abundance)	cyanobacteria + depth + pH	5	34.88	0	0.15
	area + cyanobacteria + pH + log chronic PTI	6	35.01	0.13	0.14
	area + cyanobacteria + depth + pH + log chronic PTI	7	35.05	0.17	0.14
	cyanobacteria + depth + veg disturbance + pH	6	35.41	0.53	0.11
	cyanobacteria + depth + pH + zone width	6	35.52	0.64	0.11
	cyanobacteria + depth + pH + log chronic PTI + TP	7	35.85	0.97	0.09
	cyanobacteria + depth + pH + TP	6	36.36	1.48	0.07
	cyanobacteria + depth + pH + log chronic PTI	6	36.51	1.63	0.07
	area + cyanobacteria + depth + pH	6	36.53	1.65	0.07
	area + cyanobacteria + pH + log chronic PTI + TP	7	36.89	2.01	0.05
	null	2	60.58	25.7	
	global	16	193.97	159.09	
log(Odonata Abundance)	crop type + veg disturbance + pH	5	66.47	0	0.33
	veg disturbance + pH	4	68.58	2.12	0.11
	area + crop type + veg disturbance + pH	6	69.18	2.71	0.08
	crop type + veg disturbance + pH + log chronic PTI	6	69.24	2.77	0.08
	crop type + veg disturbance + pH + zone width	6	69.31	2.85	0.08
	crop type + veg disturbance + pH + TP	6	69.46	2.99	0.07
	crop type + depth + veg disturbance + pH	6	69.62	3.15	0.07
	crop type + veg disturbance + pH + TN	6	69.89	3.42	0.06

	crop type + veg disturbance + NPOC + pH	6	69.94	3.48	0.06
	crop type + veg disturbance + percent crop + pH	6	69.98	3.51	0.06
	null	2	74.43	7.96	
	global	16	266.7	200.23	
log(Hemiptera Abundance)	depth	3	35.9	0	0.18
	cyanobacteria + depth	4	36.05	0.15	0.17
	depth + TN	4	36.65	0.76	0.12
	cyanobacteria + depth + log chronic PTI	5	37.38	1.48	0.09
	cyanobacteria + depth + veg disturbance	5	37.49	1.59	0.08
	depth + NPOC	4	37.65	1.75	0.07
	cyanobacteria + depth + TN	5	37.83	1.93	0.07
	area + depth	4	37.97	2.07	0.06
	cyanobacteria + depth + zone width	5	38.01	2.11	0.06
	depth + log chronic PTI	4	38.45	2.55	0.05
	depth + TP	4	38.45	2.55	0.05
	null	2	40.64	4.74	
	global	16	223.37	187.47	
log(Zooplankton to Macroinvertebrate Ratio)	depth + TN	4	48.94	0	0.19
	depth + pH + TN	5	49.83	0.89	0.12
	depth + pH	4	49.91	0.96	0.12
	pH	3	50.06	1.11	0.11
	NPOC + pH	4	50.5	1.55	0.09
	TN	3	50.54	1.6	0.08
	NPOC	3	50.64	1.69	0.08
	depth + TN + zone width	5	50.78	1.84	0.08
	cyanobacteria + depth + pH	5	50.83	1.89	0.07
	cyanobacteria + depth + TN	5	51.06	2.11	0.07
	null	2	51.59	2.65	
	global	16	211.55	162.61	
log(Relative Daphnia Abundance)	crop type + depth + TN + zone width	6	46.67	0	0.16
	crop type + depth + TN	5	47.06	0.39	0.13
	crop type + depth + TP	5	47.22	0.55	0.12
	crop type + depth + zone width	5	47.58	0.91	0.1
	crop type + depth + pH	5	47.6	0.92	0.1
	crop type + depth	4	47.75	1.07	0.09
	crop type + depth + NPOC	5	48.37	1.7	0.07

	crop type + depth + veg disturbance + TN	6	48.53	1.86	0.06
	crop type + depth + zone width + TP	6	48.84	2.16	0.05
	crop type + depth + percent crop	5	48.9	2.22	0.05
	area + crop type + depth + pH	6	48.9	2.23	0.05
	null	2	56.87	10.2	
	global	16	226.9	180.23	
log(Relative Copepod Abundance)	area + cyanobacteria + depth + percent crop + log chronic PTI	7	48.18	0	0.38
	area + cyanobacteria + depth + veg disturbance + percent crop + log chronic PTI	8	51.18	3	0.09
	cyanobacteria + depth + percent crop	5	51.19	3.01	0.09
	area + cyanobacteria + depth + log chronic PTI	6	51.43	3.25	0.08
	area + conductivity + cyanobacteria + depth + percent crop + log chronic PTI	8	51.6	3.42	0.07
	area + cyanobacteria + depth + percent crop + pH + log chronic PTI	8	51.68	3.49	0.07
	cyanobacteria + depth + percent crop + log chronic PTI	6	51.68	3.5	0.07
	area + cyanobacteria + depth + NPOC + percent crop + log chronic PTI	8	51.78	3.59	0.06
	depth + percent crop	4	52.17	3.98	0.05
	area + cyanobacteria + depth + percent crop + log chronic PTI + zone width	8	52.18	4	0.05
	null	2	52.35	4.17	
	global	16	225.67	177.49	
log(Relative Ostracod Abundance)	crop type + cyanobacteria + NPOC + TP	-13	46.04	0	0.26
	crop type + cyanobacteria + NPOC + pH + TP	-11	46.74	0.7	0.19
	crop type + cyanobacteria + veg disturbance + NPOC + TP	-12	47.45	1.42	0.13
	conductivity + crop type + cyanobacteria + NPOC + TP	-12	48.16	2.12	0.09
	crop type + cyanobacteria + NPOC + pH + zone width + TP	-10	48.85	2.81	0.06
	crop type + cyanobacteria + pH	-16	48.99	2.95	0.06
	crop type + cyanobacteria + NPOC + zone width + TP	-13	49.03	2.99	0.06
	conductivity + crop type + cyanobacteria + NPOC + pH	-15	49.14	3.1	0.06

	crop type + cyanobacteria + NPOC + percent crop + TP	-13	49.55	3.51	0.05
	crop type + veg disturbance + cyanobacteria + NPOC + pH + TP	-10	49.56	3.53	0.05
	null	61.9	75		
	global	237	249.75		
Relative Insect Abundance Squared	crop type + cyanobacteria + pH + log chronic PTI + zone width + TP	8	0.73	0	0.19
	cyanobacteria + pH + log chronic PTI + zone width + TP	7	1.09	0.35	0.16
	cyanobacteria + pH + log chronic PTI + TP	6	1.28	0.55	0.14
	crop type + cyanobacteria + pH + log chronic PTI + TP	7	1.57	0.84	0.12
	cyanobacteria + depth + pH + log chronic PTI + TP	7	2.32	1.59	0.09
	area + cyanobacteria + depth + pH + log chronic PTI + TP	8	2.51	1.77	0.08
	cyanobacteria + depth + pH + log chronic PTI + zone width + TP	8	2.86	2.13	0.07
	area + cyanobacteria + depth + pH + log chronic PTI	7	3.1	2.36	0.06
	cyanobacteria + pH + log chronic PTI + zone width	6	3.49	2.76	0.05
	crop + cyanobacteria + pH + log chronic PTI + zone width	7	3.53	2.8	0.05
	null	2	24.4	23.67	
	global	16	185.8	185.07	
Cube Root(Relative Snail Abundance)	conductivity + depth + percent crop + pH + log chronic PTI + TN + zone width	9	-21.76	0	0.44
	conductivity + depth + veg disturbance + percent crop + pH + log chronic PTI + TN + zone width	10	-19.18	2.58	0.12
	conductivity + depth + percent crop + log chronic PTI + TN + zone width	8	-18.89	2.87	0.11
	conductivity + depth + percent crop + pH + log chronic PTI + TN + zone width + TP	10	-18.24	3.53	0.08
	cyanobacteria + depth + veg disturbance + percent crop + pH + log chronic PTI + TN + zone width	10	-18.22	3.54	0.08
	depth + veg disturbance + percent crop + pH + log chronic PTI + TN + zone width	9	-17.47	4.29	0.05

	conductivity + cyanobacteria + depth + percent crop + pH + log chronic PTI + TN + zone width	10	-17.19	4.57	0.05
	conductivity + cyanobacteria + depth + veg disturbance + percent crop + pH + log chronic PTI + TN + zone width	11	-17.08	4.68	0.04
	depth + percent crop + pH + log chronic PTI + TN + zone width	8	-16.79	4.97	0.04
	null	2	9.88	31.64	
	global	16	139.38	161.14	
Cube Root(Relative Coleoptera Abundance)	cyanobacteria	3	-38.28	0	0.21
	cyanobacteria + depth + NPOC + TN	6	-37.98	0.31	0.18
	cyanobacteria + NPOC + TN	5	-37.18	1.1	0.12
	cyanobacteria + TN	4	-36.81	1.47	0.1
	cyanobacteria + depth + veg disturbance + NPOC + TN	7	-36.54	1.75	0.09
	cyanobacteria + veg disturbance	4	-36.06	2.22	0.07
	cyanobacteria + log chronic PTI	4	-35.96	2.32	0.06
	cyanobacteria + NPOC	4	-35.85	2.44	0.06
	cyanobacteria + veg disturbance + NPOC + TN	6	-35.8	2.48	0.06
	cyanobacteria + percent crop	4	-35.75	2.53	0.06
	null	2	-30.79	7.49	
	global	16	153.94	192.22	
Square Root(Relative Diptera Abundance)	area + cyanobacteria + depth + pH + log chronic PTI	7	-16.95	0	0.24
	area + cyanobacteria + depth + pH + log chronic PTI + TP	8	-16.12	0.82	0.16
	cyanobacteria + depth + pH + log chronic PTI + TP	7	-16.02	0.93	0.15
	area + cyanobacteria + depth + log chronic PTI	6	-15.38	1.57	0.11
	area + cyanobacteria + depth + percent crop + pH + log chronic PTI	8	-14.77	2.18	0.08
	area + cyanobacteria + pH + log chronic PTI	6	-14.14	2.8	0.06
	cyanobacteria + depth + pH + log chronic PTI + zone width + TP	8	-14.14	2.81	0.06
	area + cyanobacteria + percent crop + pH + log chronic PTI	6	-14.07	2.88	0.06
	area + cyanobacteria + log chronic PTI	5	-13.74	3.2	0.05
	area + cyanobacteria + depth + NPOC + pH + log chronic PTI	8	-13.6	3.35	0.04

	null	2	11.88	28.83	
	global	16	159.03	175.98	
Cube Root(Relative Odonata Abundance)	crop type + veg disturbance	4	-22.56	0	0.21
	crop type + veg disturbance + pH	5	-22.09	0.47	0.16
	crop type + veg disturbance + log chronic PTI	5	-21.5	1.05	0.12
	crop type +cyanobacteria + veg disturbance	5	-21.24	1.31	0.11
	crop type + veg disturbance + percent crop	5	-20.69	1.87	0.08
	veg disturbance + pH	4	-20.54	2.01	0.08
	crop type + veg disturbance + TP	5	-20.42	2.14	0.07
	depth + NPOC + TP	5	-20.14	2.42	0.06
	veg disturbance + TP	4	-20.08	2.47	0.06
	crop type + depth + veg disturbance	5	-20	2.56	0.06
	null	2	-16.91	5.65	
	global	16	175.35	197.91	
log(Relative Hemiptera Abundance)	cyanobacteria + depth + log chronic PTI + TN	6	36.12	0	0.3
	cyanobacteria + depth + TN	5	37.13	1.01	0.18
	cyanobacteria + depth + log chronic PTI + TN + zone width	7	38.33	2.21	0.1
	cyanobacteria + depth + NPOC + log chronic PTI + TN	7	38.9	2.78	0.07
	cyanobacteria + depth + NPOC + TN	6	39.12	3	0.07
	cyanobacteria + depth + TN + zone width	6	39.2	3.08	0.06
	area + cyanobacteria + depth + log chronic PTI + TN	7	39.26	3.13	0.06
	cyanobacteria + depth + veg disturbance + log chronic PTI + TN	7	39.43	3.31	0.06
	cyanobacteria + depth + pH + log chronic PTI + TN	7	39.52	3.4	0.05
	conductivity + cyanobacteria + depth + log chronic PTI + TN	7	39.57	3.45	0.05
	null	2	49.55	13.43	
	global	16	230.93	194.81	

[1]k: number of estimated parameters in the model
[2]AICc: Akaike's Information Criterion corrected for small sample sizes
[3]ΔAICc: different from AICc of the best approximating model
[4]weight: AICc weight, provided for models with ΔAICc < 5

Appendix K. Counts of zooplankton taxa and the 11 macroinvertebrate families common to more than half of the 27 Saskatchewan wetlands sampled June 2018, ordered by highest median abundance.

Invertebrate Group	Family or Taxa	Median (Range)	Average Count ± SD
zooplankton	Cladocera	15200 (600 - 510000)	48816.6 ± 100098.2
	Copepoda	4500 (100 - 65000)	11031.3 ± 14253.7
	Ostracoda	3500 (100 - 25600)	5086.7 ± 6727.0
macroinvertebrate	Chironomidae	960 (20 - 6780)	1767.9 ± 2036.2
	Corixidae	414 (20 - 4960)	839.4 ± 1047.7
	Physidae	113 (0 - 2300)	347.0 ± 596.7
	Dytiscidae	107 (7 - 1386)	217.9 ± 306.9
	Haliplidae	29 (0 - 1000)	100.1 ± 212.1
	Lestidae	24 (0 - 992)	178.5 ± 281.4
	Hydrachnidia*	13 (0 - 220)	37.3 ± 53.1
	Planorbidae	4 (0 - 2400)	209.0 ± 533.9
	Hyalellidae	2 (0 - 68200)	4060.9 ± 14254.8
	Lymnaeidae	1 (0 - 1226)	99.7 ± 275.4
	Notonectidae	1 (0 - 46)	5.7 ± 10.4

*Aquatic mites (hydrachnidia) not identified to family

Appendix L. Mid-summer PTIs calculated with pesticide concentrations detected in 27 Saskatchewan wetlands in May and June of 2018.

PTI	Median (Range)	Mean ± SD
Acute Mid-Summer PTI	0.018 (0.001 - 4.906)	0.253 ± 0.938
Chronic Mid-Summer PTI	5.034 (0.197 - 100.725)	8.606 ± 18.867

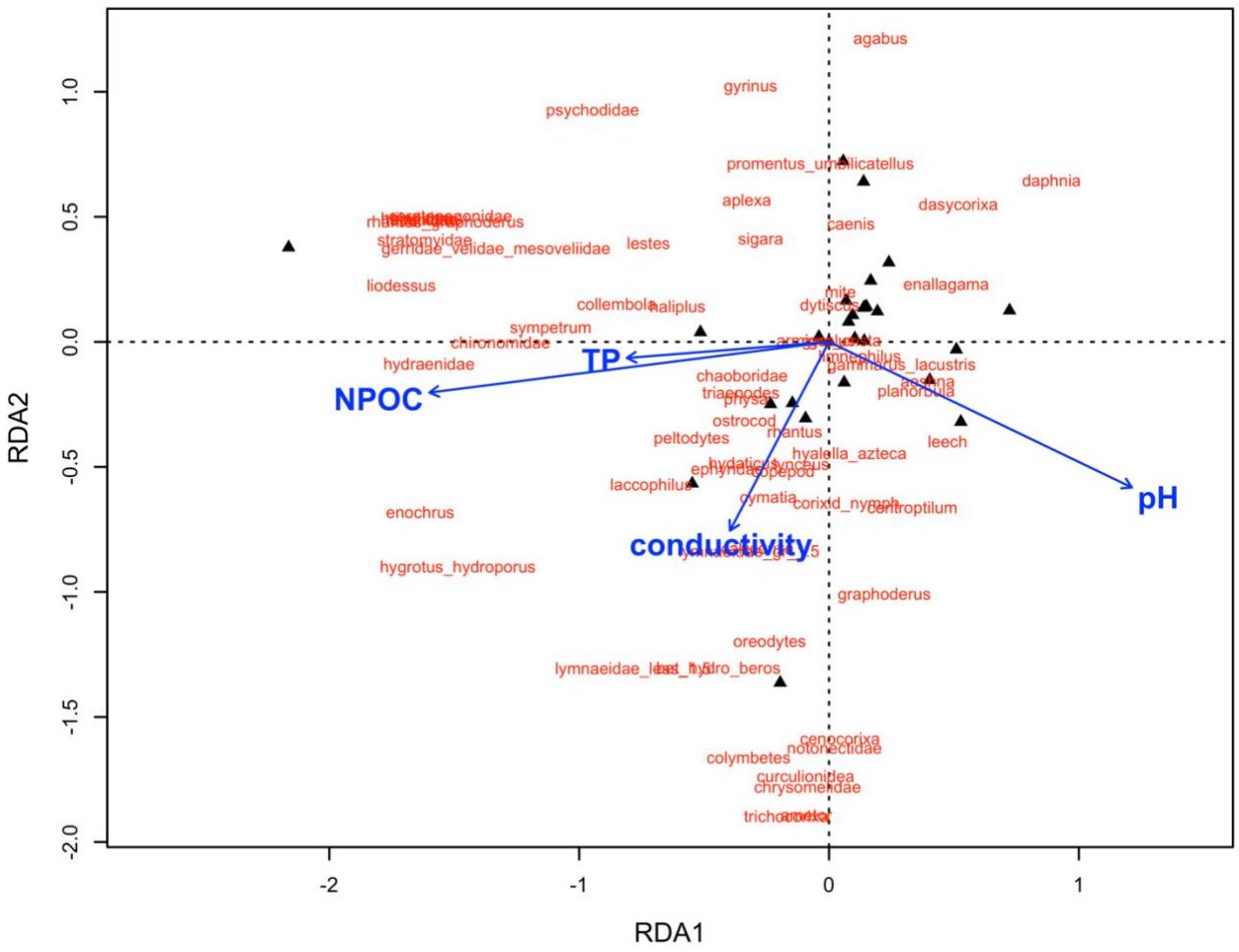
Appendix M. Effects of environmental, vegetation disturbance, water quality, and pesticide variables on aquatic invertebrate community endpoints in 27 Saskatchewan wetlands assessed in 2018, as determined by linear models and AICc model selection (Appendix I) ($p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***).

Response	Parameter	Estimate ± SE	p	
log(Macroinvertebrate Abundance)	cyanobacteria	0.417 ± 0.154	0.010	**
	pH	-0.158 ± 0.067	0.025	*
	total nitrogen	0.085 ± 0.073	0.269	
	conductivity	0.071 ± 0.064	0.290	
log(Zooplankton Abundance)	depth: shallow	-0.462 ± 0.184	0.016	*
	zone width	-0.140 ± 0.079	0.094	.
	vegetation disturbance	0.141 ± 0.087	0.121	
	total phosphorus	-0.117 ± 0.082	0.174	
	total nitrogen	-0.124 ± 0.086	0.170	
	percent crop	-0.088 ± 0.082	0.305	
	crop type: other	-0.653 ± 0.268	0.021	*
	crop type: wheat	-0.087 ± 0.166	0.618	
Richness	cyanobacteria	-4.037 ± 2.026	0.058	.
	NPOC	3.252 ± 1.076	0.004	**
	total nitrogen	-3.773 ± 1.096	0.001	**
	zone width	1.862 ± 0.820	0.031	*
	percent crop	-1.212 ± 0.761	0.133	
	conductivity	1.213 ± 0.767	0.135	
	depth: shallow	2.469 ± 1.903	0.220	
	log(chronic PTI)	0.982 ± 0.781	0.235	
Hilsenhoff Biotic Index	area	-0.254 ± 0.108	0.027	*
	conductivity	0.250 ± 0.116	0.042	*
	cyanobacteria	1.076 ± 0.357	0.004	**
	vegetation disturbance	-0.362 ± 0.148	0.020	*
	depth: shallow	0.572 ± 0.264	0.039	*
	total nitrogen	0.319 ± 0.172	0.073	.
	total phosphorus	-0.410 ± 0.188	0.034	*
	percent crop	0.211 ± 0.129	0.119	
	crop type: other	-0.216 ± 0.356	0.571	
	crop type: wheat	-0.698 ± 0.236	0.006	**
Shannon's Diversity	cyanobacteria	-0.644 ± 0.182	0.001	***
	NPOC	0.150 ± 0.084	0.093	.
	total nitrogen	-0.284 ± 0.113	0.016	*
	total phosphorus	0.158 ± 0.083	0.073	.

	depth: shallow	0.223	±	0.144	0.142	
	zone width	0.102	±	0.071	0.172	
Shannon's Evenness	cyanobacteria	-0.175	±	0.051	0.002	**
	total nitrogen	-0.099	±	0.027	0.001	**
	total phosphorus	0.058	±	0.025	0.032	*
Berger-Parker Dominance	cyanobacteria	0.211	±	0.066	0.004	**
	total nitrogen	0.138	±	0.034	0.001	***
	total phosphorus	-0.080	±	0.033	0.023	*
log(Insect Abundance)	area	-0.165	±	0.067	0.022	*
	cyanobacteria	-0.622	±	0.153	0.001	***
	pH	-0.211	±	0.059	0.002	**
	log(chronic PTI)	-0.185	±	0.071	0.016	*
log(Snail Abundance)	cyanobacteria	-1.481	±	0.428	0.001	***
	depth: shallow	0.852	±	0.311	0.009	**
	vegetation disturbance	0.492	±	0.191	0.015	*
	total nitrogen	-0.645	±	0.263	0.016	*
	zone width	0.471	±	0.165	0.006	**
	percent crop	0.259	±	0.139	0.077	.
	log(chronic PTI)	0.252	±	0.132	0.074	.
	pH	0.274	±	0.144	0.075	.
	NPOC	0.476	±	0.239	0.055	.
	block: B	1.363	±	0.400	0.002	**
	block: C	1.216	±	0.404	0.005	**
	block: D	1.572	±	0.485	0.003	**
	block: H	1.989	±	0.372	< 0.001	***
	block: J	1.266	±	0.289	< 0.001	***
log(Coleoptera Abundance)	pH	-0.140	±	0.102	0.193	
	cyanobacteria	-0.279	±	0.243	0.275	
	zone width	0.102	±	0.104	0.353	
	NPOC	0.098	±	0.104	0.371	
log(Diptera Abundance)	cyanobacteria	-1.125	±	0.233	< 0.001	***
	depth: shallow	0.426	±	0.183	0.027	*
	pH	-0.264	±	0.086	0.003	**
	area	-0.195	±	0.102	0.066	.
	log(chronic PTI)	-0.181	±	0.097	0.075	.
	vegetation disturbance	0.163	±	0.105	0.142	
	zone width	-0.128	±	0.084	0.150	
	total phosphorus	-0.145	±	0.094	0.143	
log(Odonata Abundance)	crop type: other	-1.291	±	0.513	0.020	*

	crop type: wheat	-0.738	±	0.308	0.025	*
	vegetation disturbance	-0.605	±	0.155	0.001	***
	pH	-0.383	±	0.141	0.012	*
log(Hemiptera Abundance)	depth: shallow	-0.542	±	0.188	0.006	**
	cyanobacteria	-0.385	±	0.244	0.132	
	total nitrogen	-0.107	±	0.087	0.240	
	log(chronic PTI)	-0.109	±	0.089	0.246	
	vegetation disturbance	0.128	±	0.109	0.263	
	NPOC	-0.084	±	0.088	0.361	
log(Zooplankton to Macroinvertebrate Ratio)	depth: shallow	-0.426	±	0.222	0.069	.
	total nitrogen	-0.234	±	0.114	0.051	.
	pH	0.203	±	0.113	0.088	.
	NPOC	-0.183	±	0.112	0.121	
	zone width	-0.109	±	0.106	0.332	
	cyanobacteria	-0.368	±	0.269	0.195	
log(Relative Daphnia Abundance)	crop type: other	-1.205	±	0.348	0.001	**
	crop type: wheat	-0.029	±	0.209	0.896	
	depth: shallow	-0.811	±	0.218	< 0.001	***
	total nitrogen	-0.195	±	0.100	0.066	.
	zone width	-0.169	±	0.093	0.088	.
	total phosphorus	-0.182	±	0.099	0.082	.
	pH	0.173	±	0.099	0.100	.
	NPOC	-0.153	±	0.100	0.149	
	vegetation disturbance	0.160	±	0.119	0.206	
log(Relative Copepod Abundance)	area	-0.286	±	0.113	0.019	*
	cyanobacteria	-0.752	±	0.264	0.010	**
	depth: shallow	-0.870	±	0.231	0.001	**
	percent crop	-0.244	±	0.098	0.022	*
	log(chronic PTI)	-0.307	±	0.114	0.013	*
log(Relative Ostracod Abundance)	crop type: other	-0.524	±	0.327	0.131	
	crop type: wheat	-0.949	±	0.228	< 0.001	***
	cyanobacteria	-1.458	±	0.261	< 0.001	***
	NPOC	0.298	±	0.107	0.009	**
	total phosphorus	-0.328	±	0.120	0.010	**
	pH	0.161	±	0.098	0.123	
	vegetation disturbance	-0.178	±	0.123	0.174	
Relative Insect Abundance Squared	crop type: other	0.371	±	0.140	0.013	*
	crop type: wheat	0.123	±	0.086	0.183	
	cyanobacteria	-0.831	±	0.112	< 0.001	***

	pH	-0.175	±	0.050	0.001	***
	log(chronic PTI)	-0.200	±	0.051	< 0.001	***
	zone width	-0.078	±	0.040	0.070	.
	total phosphorus	-0.111	±	0.047	0.027	*
	depth: shallow	-0.158	±	0.094	0.112	
	area	-0.088	±	0.050	0.097	.
Cube Root(Relative Snail Abundance)	conductivity	0.085	±	0.030	0.010	*
	depth: shallow	0.188	±	0.051	0.002	**
	percent crop	0.105	±	0.029	0.002	**
	pH	0.065	±	0.027	0.024	*
	log(chronic PTI)	0.078	±	0.025	0.005	**
	total nitrogen	-0.134	±	0.031	< 0.001	***
	zone width	0.107	±	0.025	< 0.001	***
Cube Root(Relative Coleoptera Abundance)	cyanobacteria	-0.172	±	0.058	0.005	**
	depth: shallow	-0.102	±	0.053	0.070	.
	NPOC	0.074	±	0.035	0.044	*
	total nitrogen	-0.069	±	0.039	0.091	.
	vegetation disturbance	0.035	±	0.026	0.202	
Square Root(Relative Diptera Abundance)	area	-0.080	±	0.036	0.038	*
	cyanobacteria	-0.482	±	0.084	< 0.001	***
	depth: shallow	0.167	±	0.068	0.021	*
	pH	-0.079	±	0.036	0.035	*
	log(chronic PTI)	-0.098	±	0.035	0.008	**
	total phosphorus	-0.068	±	0.037	0.079	.
Cube Root(Relative Odonata Abundance)	crop type: other	-0.273	±	0.104	0.0124	*
	crop type: wheat	-0.152	±	0.063	0.0217	*
	vegetation disturbance	-0.114	±	0.034	0.0014	**
	pH	-0.043	±	0.027	0.1372	
	log(chronic PTI)	0.043	±	0.031	0.1873	
	cyanobacteria	-0.108	±	0.083	0.2156	
	percent crop	0.030	±	0.027	0.2933	
log(Relative Hemiptera Abundance)	cyanobacteria	-0.760	±	0.230	0.002	**
	depth: shallow	-0.701	±	0.176	< 0.001	***
	log(chronic PTI)	-0.166	±	0.085	0.064	.
	total nitrogen	-0.255	±	0.084	0.004	**



Appendix N. Partial RDA of scaled aquatic invertebrate taxa abundances (red) and 27 wetland sites (black triangles), constrained by conductivity, non-purgeable organic carbon (NPOC), total phosphorus (TP), and pH (blue arrows). This partial RDA significantly explained 29.6% of variance (F = 2.70, p = 0.001), and both of the first RDA axes were significant, RDA1 and RDA2 (F = 5.35, p = 0.001 and F = 2.89, p = 0.005). Conductivity (F = 2.48, p = 0.039), total phosphorus (F = 1.71, p = 0.049), and NPOC (F = 4.42, p = 0.002), and pH (F = 2.21, p = 0.020) were found to have significant associations with aquatic invertebrate taxa abundances.

Appendix O. Pearson's correlation coefficients of continuous fixed effects used in global linear models of water quality parameters measured in 22 Saskatchewan wetlands in 2019.

	Area	Percent Protection
Area	1.00	0.09
Percent Protection	0.09	1.00

Appendix P. Pearson's correlation coefficients of continuous fixed effects used in the global linear model of total phosphorus measured in 18 Saskatchewan wetlands in 2019.

	Area	Percent Protection
Area	1.00	0.06
Percent Protection	0.06	1.00

Appendix Q. Model selection results for linear models to examine the effects of perennial buffer and protection variables on pesticide and water quality parameters measured in 22 Saskatchewan study wetlands (with the exception of total phosphorus measured in 18 wetlands) in the growing season of 2019. All models with ΔAICc < 5 are reported with AICc weights in addition to global and null models. Bolded models indicate those found to have lower AICc values than null models.

Response	Perennial Variable Included in Global Model	Model Structure	[1]k	[2]AICc	[3]ΔAICc	[4]weight
total nitrogen	perennial buffer	null	2	53.43	0	0.2
		depth + perennial buffer	4	54.03	0.6	0.15
		perennial buffer	3	54.37	0.94	0.12
		dept	3	54.38	0.94	0.12
		crop type	3	54.9	1.46	0.09
		area	3	55.9	2.46	0.06
		crop type + depth	4	56.57	3.14	0.04
		area + depth	4	56.93	3.5	0.03
		area + depth + perennial buffer	5	57.22	3.79	0.03
		crop type + perennial buffer	4	57.28	3.85	0.03
		depth + crop type + area + perennial buffer + field	7	72.9	19.47	
	protection	null	2	53.43	0	0.26
		depth	3	54.38	0.94	0.16
		crop type	3	54.9	1.46	0.13
		protection	3	55.68	2.24	0.09
		area	3	55.9	2.46	0.08
		depth + protection	4	56.38	2.94	0.06
		crop type + depth	4	56.57	3.14	0.05
		area + depth	4	56.93	3.5	0.05
		field	3	57.29	3.85	0.04

		field + crop type	4	57.29	3.85	0.04
		depth + crop type + area + protection + field	7	73.61	20.18	
	percent protection	depth + percent protection	4	53.13	0	0.21
		null	2	53.43	0.31	0.18
		depth	3	54.38	1.25	0.11
		crop type	3	54.9	1.77	0.09
		percent protection	3	55.05	1.92	0.08
		area	3	55.9	2.77	0.05
		area + depth + percent protection	5	56.08	2.95	0.05
		crop type + depth	4	56.57	3.45	0.04
		area + depth	4	56.93	3.81	0.03
		crop type + depth + percent protection	5	57.14	4.02	0.03
		depth + crop type + area + percent protection + field	7	72.73	19.6	
log(total phosphorus)	perennial buffer	null	2	24.58	0	0.38
		area	3	26.02	1.44	0.18
		perennial buffer	3	26.16	1.58	0.17
		area + perennial buffer	4	27.3	2.73	0.1
		depth	3	27.34	2.76	0.1
		depth + perennial buffer	4	29.15	4.57	0.04
		area + depth	4	29.31	4.74	0.04
		depth + crop type + area + perennial buffer + field	7	63.31	38.73	
	protection	protection	3	19.51	0	0.54
		area + protection	4	20.68	1.17	0.3
		depth + protection	4	22.54	3.03	0.12
		area + depth + protection	5	24.42	4.91	0.05
		null	2	24.58	5.07	
		depth + crop type + area + protection + field	7	49.45	29.94	
	percent protection	percent protected	3	23.22	0	0.33
		area + percent protected	4	24.26	1.04	0.19
		null	2	24.58	1.36	0.17
		depth + percent protected	4	24.98	1.77	0.14
		area	3	26.02	2.8	0.08
		area + depth + percent protected	5	26.79	3.57	0.05
		depth	3	27.34	4.12	0.04
		depth + crop type + area + percent protection + field	7	59.37	36.15	

potassium	perennial buffer	area + depth	4	167.5	0	0.82
		area + depth + perennial buffer	5	170.54	3.04	0.18
		null	2	177.98	10.48	
		depth + crop type + area + perennial buffer + field	7	186.66	19.16	
	protection	area + depth	4	167.5	0	0.67
		area + depth + protection	5	169.85	2.35	0.21
		null	2	177.98	10.48	
		depth + crop type + area + protection + field	7	186.65	19.15	
	percent protection	area	3	167.5	0	0.8
		area + depth + percent protection	5	170.23	2.73	0.2
		null	2	177.98	10.48	
		depth + crop type + area + percent protection + field	7	186.42	18.92	
log(Chronic PTI)	perennial buffer	crop type	3	10.15	0	0.32
		crop type + perennial buffer	4	10.82	0.68	0.23
		crop type + depth	4	11.6	1.45	0.15
		area + crop type	4	13.38	3.23	0.06
		crop type + depth + perennial buffer	5	13.55	3.4	0.06
		area + crop type + perennial buffer	5	13.91	3.76	0.05
		field	3	14.6	4.46	0.03
		field + crop type	4	14.6	4.46	0.03
		null	2	14.66	4.51	0.03
		area + crop type + depth	5	15	4.85	0.03
		depth + crop type + area + perennial buffer + field	7	27.57	17.42	
	protection	field + protection	12.4	2.29	0	0.32
		field + crop type + protection	12.4	2.29	0	0.32
		crop type + protection	5.52	2.71	0.42	0.26
		crop type + depth + protection	5.88	5.85	3.56	0.05
		area + crop type + protection	5.56	6.48	4.18	0.04
		null	2	14.66	12.37	
		depth + crop type + area + protection + field	7	14.98	12.69	
	percent protection	crop type + percent protection	4	5.29	0	0.69
		area + crop type + percent protection	5	8.33	3.04	0.15

		crop type + depth + percent protection	5	9.13	3.84	0.1
		crop type	3	10.15	4.86	0.06
		null	2	14.66	9.37	
		depth + crop type + area + percent protection + field	7	23.21	17.92	
Cube Root(Average Pesticide Concentration)	perennial buffer	field	3	-44.1	0	0.31
		field + crop type	4	-44.1	0	0.31
		field + depth	4	-42.03	2.07	0.11
		field + crop type + depth	5	-42.03	2.07	0.11
		field + perennial buffer	4	-40.48	3.61	0.05
		field + crop type + perennial buffer	5	-40.48	3.61	0.05
		area + field	4	-39.29	4.8	0.03
		area + field + crop type	5	-39.29	4.8	0.03
		depth + crop type + area + perennial buffer + field	7	-30.57	13.53	
		null	2	-29.25	14.85	
	protection	field + protection	4	-52.92	0	0.5
		field + crop type + protection	5	-52.92	0	0.5
		depth + crop type + area + protection + field	7	-40.37	12.55	
		null	2	-29.25	23.67	
	percent protection	field + percent protection	4	-46.64	0	0.34
		field + crop type + percent protection	5	-46.64	0	0.34
		field	3	-44.1	2.55	0.09
		field + crop type	4	-44.1	2.55	0.09
		field + depth	4	-42.03	4.62	0.03
		field + crop type + depth	5	-42.03	4.62	0.03
		area + field + percent protection	5	-41.99	4.65	0.03
		area + field + crop type + percent protection	6	-41.99	4.65	0.03
		depth + crop type + area + percent protection + field	7	-35.11	11.53	
		null	2	-29.25	17.39	

[1]k: number of estimated parameters in the model
[2]AICc: Akaike's Information Criterion corrected for small sample sizes
[3]ΔAICc: different from AICc of the best approximating model
[4]weight: AICc weight, provided for models with ΔAICc < 5

Appendix R. Pearson's correlation coefficients of continuous fixed effects used in global linear models of aquatic invertebrate community endpoints measured in 20 Saskatchewan wetlands in 2019.

	Area	Conductivity	pH	Potassium	Total Nitrogen	log Chronic PTI	Percent Protection
Area	1.00	0.02	0.27	0.46	0.11	-0.05	0.14
Conductivity	0.02	1.00	0.39	0.07	0.56	-0.07	0.37
pH	0.27	0.39	1.00	0.55	0.69	-0.25	0.43
Potassium	0.46	0.07	0.55	1.00	0.35	-0.12	-0.08
Total Nitrogen	0.11	0.56	0.69	0.35	1.00	0.02	0.32
log Chronic PTI	-0.05	-0.07	-0.25	-0.12	0.02	1.00	-0.38
Percent Protection	0.14	0.37	0.43	-0.08	0.32	-0.38	1.00

Appendix S. Model selection results for linear models to examine the effects of perennial buffer and protection variables on aquatic invertebrate community endpoints measured in 20 Saskatchewan study wetlands in June 2019. All models with ΔAICc < 5 are reported with AICc weights in addition to global and null models. Bolded models indicate those found to have lower AICc values than null models.

Response	Perennial Variable Included in Global Model	Model Structure	[1]k	[2]AICc	[3]ΔAICc	[4]weight
Macroinvertebrate Abundance	perennial buffer	area + perennial buffer + log chronic PTI	5	359.1	0	0.22
		area + depth + perennial buffer + log chronic PTI	6	359.18	0.08	0.22
		area + conductivity + perennial buffer + log chronic PTI	6	359.78	0.68	0.16
		area + depth + log chronic PTI	5	360.87	1.77	0.09
		area + conductivity + depth + perennial buffer + log chronic PTI	7	361.18	2.08	0.08
		area + log chronic PTI	4	361.75	2.65	0.06
		area + K + perennial buffer + log chronic PTI	6	362.42	3.33	0.04
		area + depth + log chronic PTI + TN	6	362.72	3.62	0.04
		area + depth + pH + log chronic PTI	6	363.04	3.95	0.03

		area + perennial buffer + pH + log chronic PTI	6	363.27	4.17	0.03
		area + perennial buffer + log chronic PTI + TN	6	363.27	4.17	0.03
		null	2	367.38	8.28	
		global	12	482.22	123.12	
	protection	area + depth + protection	5	357.18	0	0.32
		area + protection	4	358.94	1.76	0.13
		area + depth + protection + log chronic PTI	6	359.29	2.11	0.11
		area + conductivity + depth + protection	6	360.1	2.92	0.07
		area + depth + K + protection	6	360.52	3.34	0.06
		area + protection + log chronic PTI	5	360.56	3.39	0.06
		K + protection	4	360.72	3.54	0.05
		protection	3	360.84	3.66	0.05
		area + depth + log chronic PTI	5	360.87	3.69	0.05
		area + depth + pH + protection	6	361.02	3.84	0.05
		area + conductivity + protection	5	361.02	3.85	0.05
		null	2	367.38	10.2	
		global	12	482.51	125.33	
	percent protection	area + percent protection + log chronic PTI	5	360.13	0	0.22
		area + depth + log chronic PTI	5	360.87	0.74	0.16
		area + conductivity + percent protection + log chronic PTI	6	361.01	0.88	0.14
		area + log chronic PTI	4	361.75	1.62	0.1
		area + depth + percent protection + log chronic PTI	6	362.2	2.07	0.08
		area + percent protection	4	362.29	2.16	0.08
		area + depth + log chronic PTI + TN	6	362.72	2.59	0.06
		area + conductivity + percent protection	5	362.81	2.68	0.06
		area + depth + pH + log chronic PTI	6	363.04	2.92	0.05
		log chronic PTI	3	363.28	3.15	0.05
		null	2	367.38	7.25	
		global	12	486.52	126.39	
Zooplankton Abundance	perennial buffer	area + depth + K + perennial buffer	6	427.5	0	0.21

	area + depth + perennial buffer	5	427.85	0.35	0.18
	area + perennial buffer	4	428.8	1.3	0.11
	area + depth + K + perennial buffer + TN	7	428.86	1.36	0.11
	area + depth + K + perennial buffer + pH	7	429.03	1.53	0.1
	area + perennial buffer + TN	5	429.35	1.85	0.08
	area + conductivity + perennial buffer	5	430.11	2.61	0.06
	area +conductivity + depth + perennial buffer	6	430.31	2.81	0.05
	area + depth + perennial buffer + TN	6	430.56	3.07	0.05
	area + conductivity + depth + K + perennial buffer	7	430.57	3.07	0.05
	null	2	433.83	6.33	
	global	12	529.86	102.36	
protection	area + depth + K	5	432.69	0	0.16
	area + depth	4	433.26	0.57	0.12
	area + depth + protection	5	433.35	0.66	0.12
	depth	3	433.38	0.69	0.12
	protection	3	433.55	0.86	0.11
	depth + protection	4	433.65	0.96	0.1
	null	2	433.83	1.14	0.09
	area + depth + K + protection	6	434.49	1.8	0.07
	area + protection	4	434.49	1.8	0.07
	area	3	434.86	2.17	0.05
	global	12	546.28	113.59	
percent protection	area + percent protection	4	431.01	0	0.18
	area + conductivity + crop type + percent protection	6	431.45	0.44	0.14
	percent protection	3	431.48	0.47	0.14
	percent protection + pH	4	432.02	1.01	0.11
	area + depth + percent protection	5	432.68	1.67	0.08
	area + depth + K	5	432.69	1.68	0.08
	percent protection + TN	4	432.74	1.73	0.07
	area + percent protection + TN	5	432.75	1.74	0.07
	area + conductivity + percent protection	5	432.82	1.81	0.07
	conductivity + crop type + percent protection	5	432.84	1.83	0.07

		null	2	433.83	2.82	
		global	12	540.87	109.86	
Richness	perennial buffer	crop type + perennial buffer	4	140.8	0	0.23
		null	2	141.66	0.86	0.15
		crop type	3	141.81	1.02	0.14
		crop type + depth + perennial buffer	5	142.13	1.33	0.12
		perennial buffer	3	142.79	1.99	0.09
		conductivity + crop type	4	143.79	2.99	0.05
		area + crop type + perennial buffer	5	143.83	3.03	0.05
		conductivity + crop type + perennial buffer	5	144.18	3.38	0.04
		depth	3	144.19	3.39	0.04
		area	3	144.19	3.4	0.04
		pH	3	144.21	3.41	0.04
		global	12	269.19	128.39	
	protection	null	2	141.66	0	0.2
		crop type	3	141.81	0.15	0.19
		crop type + protection	4	141.94	0.28	0.17
		protection	3	143.15	1.48	0.1
		conductivity + crop type	4	143.79	2.12	0.07
		depth	3	144.19	2.53	0.06
		area	3	144.19	2.53	0.06
		pH	3	144.21	2.54	0.06
		crop type + depth + protection	5	144.23	2.57	0.06
		conductivity	3	144.3	2.64	0.05
		global	12	271.89	130.23	
	percent protection	null	2	141.66	0	0.23
		crop type	3	141.81	0.15	0.21
		conductivity + crop type	4	143.79	2.12	0.08
		percent protection	3	143.82	2.15	0.08
		crop type + percent protection	4	143.86	2.19	0.08
		depth	3	144.19	2.53	0.07
		area	3	144.19	2.53	0.07
		pH	3	144.21	2.54	0.06
		conductivity	3	144.3	2.64	0.06
		TN	3	144.34	2.67	0.06
		global	12	272.08	130.42	
		conductivity	3	4.26	0	0.13

		pH	3	4.55	0.29	0.11
		null	2	4.56	0.31	0.11
		log chronic PTI	3	4.7	0.44	0.11
		conductivity + log chronic PTI	4	4.74	0.49	0.1
	perennial buffer	conductivity + perennial buffer + log chronic PTI	5	4.8	0.54	0.1
		conductivity + perennial buffer	4	4.96	0.7	0.09
		K	3	5.02	0.76	0.09
		conductivity + K	4	5.08	0.82	0.09
		conductivity + K + perennial buffer	5	5.59	1.33	0.07
		global	12	127.62	123.36	
		conductivity	3	4.26	0	0.13
		pH	3	4.55	0.29	0.11
		null	2	4.56	0.31	0.11
		log chronic PTI	3	4.7	0.44	0.1
		conductivity + log chronic PTI	4	4.74	0.49	0.1
Hilsenhoff Biotic Index	protection	K	3	5.02	0.76	0.09
		conductivity +K	4	5.08	0.82	0.09
		K + log chronic PTI	4	5.81	1.56	0.06
		pH + log chronic PTI	4	5.95	1.69	0.06
		conductivity + pH	4	6.13	1.87	0.05
		global	12	130.3	126.04	
		conductivity	3	4.26	0	0.14
		pH	3	4.55	0.29	0.12
		null	2	4.56	0.31	0.12
		log chronic PTI	3	4.7	0.44	0.12
		conductivity + log chronic PTI	4	4.74	0.49	0.11
	percent protection	K	3	5.02	0.76	0.1
		conductivity + K	4	5.08	0.82	0.1
		K + log chronic PTI	4	5.81	1.56	0.07
		pH + log chronic PTI	4	5.95	1.69	0.06
		conductivity + percent protection + log chronic PTI	5	5.95	1.69	0.06
		global	12	129.66	125.4	
		K + perennial buffer	4	30.68	0	0.24
		perennial buffer	3	31.66	0.99	0.14
Shannon's Diversity	perennial buffer	K	3	32.18	1.51	0.11
		null	null	32.31	1.63	0.1
		perennial buffer + pH	4	32.74	2.06	0.08

		conductivity + K + perennial buffer	5	32.97	2.3	0.07
		depth + K + perennial buffer	5	33.01	2.34	0.07
		K + TN	4	33.35	2.67	0.06
		conductivity + perennial buffer	4	33.57	2.9	0.06
		K + perennial buffer + TN	5	33.67	3	0.05
		global	12	149.13	118.45	
	protection	K	3	32.18	0	0.22
		null	2	32.31	0.13	0.2
		K + TN	4	33.35	1.16	0.12
		K + protection	4	34.08	1.9	0.08
		protection	3	34.38	2.2	0.07
		pH	3	34.65	2.47	0.06
		TN	3	34.7	2.52	0.06
		depth	3	34.75	2.57	0.06
		log chronic PTI	3	34.85	2.67	0.06
		area + K	4	34.94	2.76	0.05
		global	12	152.19	120.01	
	percent protection	K	3	32.18	0	0.21
		null	2	32.31	0.13	0.2
		K + TN	4	33.35	1.16	0.12
		percent protection	3	33.85	1.67	0.09
		K + percent protection	4	34.23	2.05	0.08
		pH	3	34.65	2.47	0.06
		TN	3	34.7	2.52	0.06
		depth	3	34.75	2.57	0.06
		log chronic PTI	3	34.85	2.67	0.06
		area + K	4	34.94	2.76	0.05
		global	12	152.28	120.1	
Shannon's Evenness	perennial buffer	K + TN	4	-19.82	0	0.19
		K + perennial buffer	4	-19.48	0.34	0.16
		K	3	-19.2	0.62	0.14
		conductivity + K + TN	5	-18.97	0.85	0.12
		conductivity + K + perennial buffer + TN	6	-18.2	1.62	0.08
		K + perennial buffer + TN	5	-18	1.81	0.08
		null	2	-17.95	1.87	0.07
		perennial buffer	3	-17.44	2.38	0.06
		area + K + TN	5	-17.29	2.53	0.05

		K + pH + TN	5	-17.13	2.69	0.05
		global	12	92.02	111.84	
	protection	K + TN	4	-19.82	0	0.24
		K	3	-19.2	0.62	0.18
		conductivity + K + TN	5	-18.97	0.85	0.16
		null	2	-17.95	1.87	0.1
		area + K + TN	5	-17.29	2.53	0.07
		K + pH + TN	5	-17.13	2.69	0.06
		area + K	4	-16.73	3.08	0.05
		K + log chronic PTI + TN	5	-16.48	3.33	0.05
		pH + TN	4	-16.42	3.4	0.04
		K + log chronic PTI	4	-16.41	3.4	0.04
		global	12	94.87	114.69	
	percent protection	K + TN	4	-19.82	0	0.23
		K	3	-19.2	0.62	0.17
		conductivity + K + TN	5	-18.97	0.85	0.15
		null	2	-17.95	1.87	0.09
		area + K + TN	5	-17.29	2.53	0.07
		K + pH + TN	5	-17.13	2.69	0.06
		area + K	4	-16.73	3.08	0.05
		K + percent protection	4	-16.58	3.24	0.05
		K + log chronic PTI + TN	5	-16.48	3.33	0.04
		pH + TN	4	-16.42	3.4	0.04
		K + log chronic PTI	4	-16.41	3.4	0.04
		global	12	93.52	113.34	
Berger-Parker Dominance	perennial buffer	null	2	-6.87	0	0.22
		perennial buffer	3	-6.23	0.64	0.16
		K	3	-5.58	1.29	0.12
		K + perennial buffer	4	-5.06	1.81	0.09
		perennial buffer + pH	4	-4.88	2	0.08
		pH	3	-4.63	2.25	0.07
		log chronic PTI	3	-4.5	2.37	0.07
		depth	3	-4.29	2.59	0.06
		area	3	-4.22	2.65	0.06
		conductivity	3	-4.19	2.69	0.06
		global	12	116.03	122.9	
	protection	null	2	-6.87	0	0.27
		K	3	-5.58	1.29	0.14
		pH	3	-4.63	2.25	0.09

		log chronic PTI	3	-4.5	2.37	0.08
		depth	3	-4.29	2.59	0.07
		protection	3	-4.28	2.6	0.07
		area	3	-4.22	2.65	0.07
		conductivity	3	-4.19	2.69	0.07
		TN	3	-4.13	2.74	0.07
		area + K	4	-3.61	3.26	0.05
		global	12	120.57	127.44	
	percent protection	null	2	-6.87	0	0.27
		K	3	-5.58	1.29	0.14
		percent protection	3	-4.66	2.22	0.09
		pH	3	-4.63	2.25	0.09
		log chronic PTI	3	-4.5	2.37	0.08
		depth	3	-4.29	2.59	0.07
		area	3	-4.22	2.65	0.07
		conductivity	3	-4.19	2.69	0.07
		TN	3	-4.13	2.74	0.07
		area + K	4	-3.61	3.26	0.05
		global	12	117.21	124.08	
Insect Abundance	perennial buffer	conductivity + K + perennial buffer	5	365.77	0	0.19
		conductivity + perennial buffer	4	366.11	0.33	0.16
		area + conductivity + perennial buffer	5	366.72	0.95	0.12
		depth	3	367.32	1.54	0.09
		area + conductivity + depth + perennial buffer	6	367.44	1.67	0.08
		conductivity + depth + perennial buffer	5	367.52	1.75	0.08
		conductivity + depth	4	367.57	1.79	0.08
		conductivity	3	367.79	2.02	0.07
		conductivity + perennial buffer + pH	5	367.83	2.06	0.07
		null	2	367.99	2.22	0.06
		global	12	492.18	126.41	
	protection	conductivity + K + protection	5	366.45	0	0.06
		pH + protection	4	366.56	0.1	0.06
		conductivity + protection	4	367.06	0.61	0.04
		depth	3	367.32	0.86	0.04
		conductivity + pH + protection	5	367.38	0.92	0.04

	conductivity + depth + protection	5	367.56	1.1	0.03
	conductivity + depth	4	367.57	1.11	0.03
	conductivity	3	367.79	1.34	0.03
	depth + pH + protection	5	367.99	1.53	0.03
	null	2	367.99	1.53	0.03
	global	12	493.32		
percent protection	conductivity + percent protection	4	366.74	0	0.16
	depth	3	367.32	0.57	0.12
	conductivity + percent protection + pH	5	367.45	0.7	0.11
	conductivity + depth	4	367.57	0.82	0.11
	conductivity	3	367.79	1.05	0.1
	conductivity + K + percent protection	5	367.96	1.21	0.09
	null	2	367.99	1.25	0.09
	K	3	368.17	1.43	0.08
	area + conductivity + percent protection	5	368.24	1.5	0.08
	conductivity + K	4	368.32	1.58	0.07
	global	12	496.19	129.45	

[1]k: number of estimated parameters in the model
[2]AICc: Akaike's Information Criterion corrected for small sample sizes
[3]ΔAICc: different from AICc of the best approximating model

Appendix T. Effects of environmental, perennial buffer or protection variables on pesticide and water quality parameters in 22 Saskatchewan wetlands (with the exception of total phosphorus measured in 18 wetlands) measured in 2019, as determined by linear models and AICc model selection (Appendix P) ($p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***).

Response	Perennial Variable Included in Global Model	Parameter	Estimate ± SE	p	
log(Total Phosphorus)	protection	protection: protected	-0.504 ± 0.167	0.005	**
		area	0.179 ± 0.128	0.200	
Potassium	perennial buffer	area	10.139 ± 3.364	0.007	**
		depth: shallow	14.969 ± 3.995	0.001	**
	protection	area	10.139 ± 3.364	0.007	**
		depth: shallow	14.969 ± 3.995	0.001	**
	percent protection	area	10.139 ± 3.364	0.007	**
		depth: shallow	14.969 ± 3.995	0.001	**

log(Chronic PTI)	perennial buffer	crop type: other	-0.821	±	0.240	0.001	**
		crop type: wheat	-0.669	±	0.245	0.010	*
		perennial buffer: present	-0.284	±	0.185	0.151	
		depth: shallow	0.234	±	0.182	0.229	
	protection	block: G	-0.036	±	0.156	0.834	
		block: M	0.197	±	0.173	0.294	
		block: Q	0.446	±	0.154	0.008	**
		block: R	-0.219	±	0.156	0.199	
		block: S	-0.322	±	0.162	0.067	.
		protection: protected	-0.520	±	0.132	< 0.001	***
		crop type: other	-0.669	±	0.195	0.001	**
		crop type: wheat	-0.607	±	0.192	0.003	**
	percent protection	crop type: other	-0.746	±	0.181	0.001	***
		crop type: wheat	-0.501	±	0.141	0.002	**
		percent protection	-0.004	±	0.001	0.010	*
Cube Root(Average Pesticide Concentration)	perennial buffer	block: G	0.012	±	0.158	0.946	
		block: M	0.467	±	0.178	0.015	*
		block: Q	0.690	±	0.158	< 0.001	***
		block: R	-0.051	±	0.158	0.766	
		block: S	-0.270	±	0.166	0.132	
		crop type: other	NA	±	NA	NA	
		crop type: wheat	NA	±	NA	NA	
	protection	block: G	0.093	±	0.121	0.481	
		block: M	0.467	±	0.134	0.001	**
		block: Q	0.690	±	0.119	< 0.001	***
		block: R	0.030	±	0.121	0.819	
		block: S	-0.293	±	0.125	0.031	*
		protection: protected	-0.348	±	0.096	0.001	***
		crop type: other	NA	±	NA	NA	
		crop type: wheat	NA	±	NA	NA	
	percent protection	block: G	0.036	±	0.138	0.810	
		block: M	0.453	±	0.154	0.007	**
		block: Q	0.747	±	0.139	< 0.001	***
		block: R	-0.026	±	0.138	0.860	
		block: S	-0.241	±	0.145	0.126	
		percent protection	-0.268	±	0.108	0.022	*
		crop type: other	NA	±	NA	NA	
		crop type: wheat	NA	±	NA	NA	