Agriculture and the Life Aquatic:

Effects of Agricultural Landscape Structure on Farmland Aquatic Biodiversity and Water Quality

by

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in

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Abstract

Agriculture is associated with many of the leading threats to freshwater ecosystems, which are the most threatened environments on Earth. The overarching goal of my dissertation was to advance our understanding of the relationships between agricultural landscape structure and aquatic biodiversity and water quality, to identify potential management options for farmland aquatic ecosystems. In Chapter 2, I investigated the responses of anurans to agricultural landscape structure, with the particular goal of testing predictions about the influence of farmland compositional and configurational heterogeneity. I found that, while forest cover was the strongest predictor of anuran richness and abundance, farmland configurational heterogeneity was also positively related to abundance. In Chapter 3, I investigated the direct and indirect relationships between agricultural landscape structure and different physicochemical and biological measures of water quality in farmland drainage ditches. I found that physicochemical water quality was most strongly related to landscape composition, specifically, amounts of forest cover and high-intensity crop cover in the surrounding landscape. I also found support for a positive direct relationship between a biological measure of water quality, macroinvertebrate richness, and configurational heterogeneity, and indirect relationships between macroinvertebrate richness and landscape composition. In Chapter 4, I examined whether macroinvertebrate traits can be used as reliable indicators of elevated levels of specific agrichemical pollutants in farmland drainage ditches. My results suggested that macroinvertebrate indicators are probably less efficient than rigorous chemical sampling for the monitoring of specific contaminants in farm wetlands. Taken together, my results suggest that landscape management to support farmland aquatic ecosystems should focus

mainly on landscape composition; in particular, agri-environmental policies should aim to increase or maintain amounts of non-crop cover such as forest, and reduce or maintain low amounts of high-intensity crop cover. My results also suggest that increasing farmland configurational heterogeneity, through reducing crop field sizes/increasing edge density, can be an effective management option to enhance aquatic biodiversity in agricultural landscapes. In addition, my results indicate a need for more management actions in our region to reduce nutrient pollution, and stronger policies for routine water quality data collection, as well as establishment and enforcement of water quality standards.

Preface

Co-authorship Statement

This thesis is formatted using the integrated thesis format, and therefore each data chapter was written as an independent manuscript. Chapters 2 and 3 have been published in, and Chapter 4 has been submitted to, a peer-reviewed journal when this thesis was completed. The text of each published chapter has been reproduced in the following thesis in whole, but with minor changes to formatting. There is some repetition in the introductions, methods and discussions; however, I have cross-referenced between chapters to reduce repetition as much as possible.

Each data chapter is a co-authored work; however, I performed the majority of the work presented in this thesis. I proposed and developed all research questions and hypotheses, in cooperation with my supervisor, Dr. Lenore Fahrig. I was primarily responsible for the design of projects to address these research questions. I carried out all field work and collected the majority of the data for Chapters 2, 3 and 4. I analysed all of the data and wrote all first drafts of the data chapters. The contributions of my co-authors are as follows:

(1) Dr. Lenore Fahrig (Carleton University) contributed to all data chapters, helping to develop research questions and hypotheses. She also provided guidance during project design, and contributed significantly to the writing of each chapter.

(2) Dr. Gregory Mitchell (National Wildlife Research Center) contributed to Chapter 3. He provided significant guidance during the statistical analysis of the data and contributed to the writing of this chapter.

(3) Lindsay Bellingham contributed to Chapter 3. She contributed to the design of the *Ceriodaphnia dubia* laboratory bioassays and was the primary researcher who conducted those experiments and collected the *C. dubia* population data.

I have author rights from the publishing company that grant me permission to reproduce the two published manuscripts in this thesis. I have also received permission from my co-authors Lenore Fahrig, Gregory Mitchell, and Lindsay Bellingham to use the work in this thesis. A specific chapter that has been published elsewhere must be cited using the journal citation information provided below. However, to reference my thesis as a whole or an unpublished chapter, I recommend using the following citation:

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This dissertation is dedicated with love to my mother,

Margaret R. Collins

And to the memory of my father,

Richard E. Collins

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Chapter 1 – General Introduction

Freshwater ecosystems are facing greater threats that any other environment (Millennium Ecosystem Assessment, 2005; WWF, 2018). While covering < 1% of the Earth, they harbour almost 10% of all known animal species, including 35% of vertebrates (Balian et al., 2008), and are experiencing faster population declines than terrestrial or marine systems (WWF, 2018). Aquatic systems and freshwater biodiversity are particularly sensitive to impacts associated with human activities, such as land use change (Reid et al., 2018; WWF, 2018). Furthermore, despite facing disproportionately high levels of threat, taxonomic biases in research and published literature imply that most freshwater biodiversity is underrepresented in conservation efforts (Hecnar, 2009; Donaldson et al., 2016; Di Marco et al., 2017).

Of all human activities, agriculture is associated with most of the largest impacts on freshwater. Agricultural activities have significantly altered global hydrological cycles and biodiversity patterns through land use change, widespread contamination, and unsustainable water consumption (Blann et al., 2009; Gordon et al., 2010; Hoekstra and Mekonnen, 2012). It has been estimated that agricultural production accounts for 92% of humanity's global water footprint, defined as the amount of water consumed or polluted (Hoekstra and Mekonnen, 2012). Ironically, the unsustainable use of water in agriculture threatens food security, as water scarcity is increasing in many places (FAO, 2011). Managing freshwater to meet production demands sustainably is therefore a critical global priority, and major challenge (Gordon et al., 2010).

Land conversion for agriculture is a leading cause of freshwater habitat loss and modification (United Nations Development Programme, 2000; Millennium Ecosystem

Assessment, 2005; Blann et al., 2009). A common type of landscape change for agriculture is land drainage. At least 25% of agricultural land in the midwestern United States, for example, is artificially drained, with some states exceeding 50% (Pavelis, 1987). Drainage is implemented to remove excess water from fields for successful crop growth, and is achieved using surface and subsurface methods (Pavelis, 1987; Blann et al., 2009). Land drainage results in extensive wetland loss, physical modification of aquatic habitats, and increased rates of pollutant loadings on aquatic systems (Blann et al., 2009; Schilling et al., 2015). For example, subsurface drains significantly reduce groundwater travel time and the potential for runoff remediation via water infiltration through soil and riparian buffers (Schilling et al., 2015).

Widespread agrichemical contamination is also a major threat to freshwater ecosystems (Camargo and Alonso, 2006; Relyea, 2009; Ippolito et al., 2015; Stehle and Schulz, 2015). Commonly used agrichemicals such as insecticides, herbicides, and excess nutrients from fertilizers enter aquatic systems via surface runoff, groundwater leaching, and spray drift, where they can alter ecosystem functioning and pose significant toxicological risks to aquatic organisms (Camargo and Alonso, 2006; Relyea, 2009). Nitrogen, for example, is regarded as one of the most ubiquitous and significant freshwater chemical contaminants in the world (Spalding and Exner, 1993; Camargo and Alonso, 2006). An estimated > 140 million tonnes of nitrogen was applied to agricultural lands in 2016 over the globe (FAO, 2019). Elevated levels in aquatic systems are toxic to biota and also result in eutrophication and hypoxia (Camargo and Alonso, 2006). Pesticide levels in surface waters are also primary causes of ecosystem impairment (Stehle and Schulz, 2015). It was estimated that almost 6 million tonnes of pesticides

were applied to farmlands in 2016 (FAO, 2018), with most nearby freshwater habitats facing high risks of contamination. For example, water bodies within 40% of the global land surface have been estimated to be at risk of insecticide contamination from runoff (Ippolito et al., 2015), and levels in surface waters across the globe have been shown to frequently exceed regulatory thresholds (Stehle and Schulz, 2015).

Aquatic ecosystems are influenced by the composition and configuration of cover types in the surrounding terrestrial landscape. Landscape composition influences aquatic biodiversity through certain cover types providing allochthonous resources to aquatic food webs (Wallace et al., 1997), or by providing habitat for the terrestrial life stages of some taxa (Hecnar and M'Closkey, 1998). Landscape composition also influences water quality through certain cover types acting as agrichemical sources or sinks (Näsholm et al., 1998; Allan and Castillo, 2007). Although less well understood, there is also evidence to suggest that the spatial patterning of cover types in the surrounding landscape, i.e. landscape configuration, influences aquatic biodiversity and water quality. For example, field sizes in agricultural landscapes may influence the degree of landscape complementation for some taxa (Fahrig et al., 2011; Fahrig et al., 2015), and surface water runoff patterns (Uuemaa et al., 2007).

Understanding the relationships between landscape structure and biological responses may suggest options for the sustainable management of agroecosystems. In particular, increasing farmland heterogeneity, i.e. the spatial complexity of the production cover types, has been suggested as a promising landscape management option to increase biodiversity in agricultural landscapes without taking working land out of production (Fahrig et al., 2011; Fahrig et al., 2015). Landscape heterogeneity is associated with

landscape multifunctionality (van der Plas et al., 2019) and is recognized as an important factor influencing population dynamics and regional biodiversity patterns (Kerr, 2001; Fahrig and Nuttle, 2005). Farmland heterogeneity can be enhanced within a given agricultural landscape by increasing the diversity of crop types grown, i.e. compositional heterogeneity, and by increasing the structural complexity of their arrangement, i.e. configurational heterogeneity (Fahrig et al., 2011).

Eastern Ontario is an agriculture-dominated region that has undergone extensive land use change for the creation of farmland (City of Ottawa, 2011; DUC, 2010). Characterized by a relatively flat topography and many areas of low-permeability soils, the region was originally dominated by wetlands and wet forests (City of Ottawa, 2011; DUC, 2010). Such characteristics promoted fertile soils, but excess water needed to be drained off the land to successfully farm, resulting in a loss of approximately 70% of pre-European settlement wetlands since the advent of commercial farming in the late 18th century (City of Ottawa, 2011; DUC, 2010). This drainage was accomplished using systems of subsurface tile drains and open constructed drains, i.e. drainage ditches. Networks of drainage ditches have been established in Eastern Ontario for at least 150 years (Irwin, 1989) and are now ubiquitous features across the region.

While generally regarded as hydrologic infrastructures of agriculture, ditches are also wetland habitats that support aquatic biota (Verdonschot et al., 2011) and provide important ecosystem services in farmland, such as flood and erosion control (Levavasseur et al., 2012), groundwater recharge (Dages et al., 2009), and water purification (Moore et al., 2001). Ditches therefore have potentially high ecological value in regions such as Eastern Ontario where much of the original wetland cover has been lost; however, they

also have the potential to be highly degraded systems, with high levels of agricultural pollutants. Thus, there is a need to understand how to best manage these modified wetlands to best support aquatic biodiversity and water quality in farmland (Dollinger et al., 2015). The Eastern Ontario context also highlights the challenge of monitoring water quality and determining the extent of freshwater contamination across an area where virtually all surface water is, to some degree, impacted by agriculture.

The objective of this thesis was to examine relationships between agricultural landscape structure and aquatic biodiversity and water quality, to identify potential management options for farmland aquatic systems. To do this, I first investigated the influence of landscape composition and configuration on anurans in agricultural landscapes, with the particular goal of testing predictions about the influence of farmland heterogeneity, as measured by crop diversity (compositional heterogeneity) and mean field size (configurational heterogeneity). I found that anuran species richness and total abundance were positively related to the amount of forest cover in agricultural landscapes, and, while forest was the strongest predictor of richness and abundance, farmland configurational heterogeneity was also positively related to anuran total abundance. These results suggest that agri-environmental policies that maintain or enhance forest cover where possible, and that promote reduction of crop field sizes, would help to maintain and enhance anuran richness and/or abundance in farmland.

In the next chapter of my thesis, I investigated the direct and indirect relationships between agricultural landscape structure and different physicochemical and biological measures of water quality in farmland drainage ditches. I found that water quality was most strongly related to measures of landscape composition: pesticides decreased as

surrounding forest cover increased, and nitrogen increased with increasing amounts of high-intensity crop cover. Crop cover was also indirectly negatively related to aquatic macroinvertebrate richness through its effects on nitrogen and dissolved oxygen levels. While there were no effects of landscape configuration on agrichemical levels, there was some support for a positive relationship between macroinvertebrate richness and configurational heterogeneity, as measured by field edge cover. These results suggest that agri-environmental policies to reduce agrichemical contamination of farmland water bodies should aim to maintain or increase amounts of non-crop cover such as forest, and reduce amounts of crop cover with high agrichemical inputs. Also, policies that increase configurational heterogeneity by increasing the amount of field edge cover will benefit aquatic macroinvertebrate richness in farmland water bodies. My results from this chapter also suggested that aquatic macroinvertebrate richness is strongly impacted by fertilizer use in this region through direct and indirect pathways, and that richness was a more sensitive water quality indicator than the other biological measures of water quality I tested, which were leaf litter decomposition and *C. dubia* population responses in bioassays.

The results of Chapter 3 led me to further investigate the potential use of aquatic macroinvertebrates as water quality indicators, while considering what makes certain taxa sensitive, and others tolerant, to particular chemical contaminants. Therefore, in Chapter 4 of my thesis, I examined whether macroinvertebrate family-level traits can be used as simple, reliable indicators of elevated levels of specific agrichemical pollutants in farmland drainage ditches, to potentially reveal areas in need of further chemical monitoring, and thus increase the coverage of monitoring across the study area, while

reducing the need for frequent, costly testing at all potentially-impacted sites. I found that two traits, oxygen acquisition and dispersal mode, were significantly associated with family-level sensitivities to nitrate and glyphosate levels, respectively. However, inspection of these relationships revealed that the responses lacked enough consistency to be reliable, chemical-specific indicators. Instead, a taxa-level, post-hoc analysis indicated that further work should be conducted to determine whether there are individual taxa whose presence at a site is a strong indicator of a lack or low levels of certain contaminants. Overall, however, these results combined with previous work suggest that we are unlikely to find chemical-specific indicators based on macroinvertebrates that are more efficient than a rigorous chemical sampling scheme. Instead, these findings suggest that water quality management efforts should focus on developing more cost-efficient chemical testing methods for the monitoring of specific contaminants in agriculture-dominated areas.

Chapter 2 – Responses of anurans to composition and configuration of agricultural landscapes

2.1 Abstract

It is imperative to identify farming systems that support biodiversity. Amphibians are the most threatened class of vertebrates globally and are particularly sensitive to the impacts of agricultural intensification. While it is known that areas of natural cover are important for amphibians in farmland, it is unknown whether cropped areas of the landscape can be structured in ways that benefit them. We examine relationships between anurans (frogs and toads) and farmland heterogeneity (structural complexity of cropped areas). We hypothesize that anurans benefit from higher compositional and configurational heterogeneity via increased prey resources and refuge habitat, and facilitation of movement. We measure compositional heterogeneity as crop diversity and configurational heterogeneity as mean field size in agricultural landscapes. We predicted that anuran richness and abundance are positively related to crop diversity and negatively related to mean field size. We surveyed 34 agricultural landscapes in eastern Ontario, Canada, representing gradients in farmland heterogeneity, for anuran richness and abundance. We used a multi-model inference approach to calculate and compare model-weighted mean coefficients to determine the direction and relative importance of landscape variables on anuran response variables. While species richness and abundance were most strongly related to the amount of forest in the landscapes, anuran abundance was also negatively related to mean field size (i.e. positive association with configurational heterogeneity). In addition, the presence of one species, the American toad, was positively associated with crop diversity. Our results suggest that conserving

natural habitats such as forest is the most effective means of maintaining anuran diversity and abundance in agricultural landscapes, but that increasing the landscape configurational heterogeneity through reduction of crop field sizes can provide an additional strategy to enhance anuran abundance.

2.2 Introduction

Effects of agriculture on biodiversity are complex. Many species depend on farmland for habitat (Javorek and Grant, 2010), and traditional agricultural systems can support high levels of biodiversity and ecosystem services (Tscharntke et al., 2005; Stoate et al., 2009; Wright et al., 2012; Baudron and Giller, 2014). However, since the second half of the 20th century there has been a shift from diverse, low-intensity systems to industrial agriculture characterized by reliance on high inputs of synthetic chemicals and homogenization of the farm landscape to achieve high yields (Stoate et al., 2001; Horrigan et al., 2002; Benton et al., 2003; Thiere et al., 2009). These systems are associated with deterioration of soil, air, and water quality, and biodiversity declines across taxa (Mclaughlin and Mineau, 1995; Stoate et al., 2001; Benton et al., 2003). Given that farmland has the potential to support biodiversity, and that the pressure to increase production will continue to intensify with increases in human population and economic growth, it is imperative that we identify farming systems that can support biodiversity while meeting agricultural demands.

It has been suggested that promoting landscape heterogeneity in agricultural systems may be critical for supporting biodiversity in farmland (Benton et al., 2003; Fahrig et al., 2011). Landscape heterogeneity increases with the number and evenness of different cover types in the landscape and with the complexity of their spatial patterning

(Fahrig and Nuttle, 2005). One method to enhance landscape heterogeneity in agricultural landscapes is to increase the diversity and pattern complexity of the more natural cover types, such as wooded areas, wetlands, and various types of vegetated field margins (Fahrig et al., 2011). As these elements provide habitat for various taxa, biodiversity is often positively associated with the amount of more natural cover in agricultural landscapes (Porej et al., 2004; Le Féon et al., 2010; Pluess et al., 2010). Many studies examining biodiversity - agricultural heterogeneity relationships have focused on this component, such that landscape heterogeneity is considered highest in landscapes with the greatest areas of natural cover (Thies et al., 2003).

Another way to enhance landscape heterogeneity in agricultural systems is to increase the spatial heterogeneity of the cropped areas of farmland by increasing the diversity and pattern complexity of the arable cover types (Fahrig et al., 2011). Relationships between biodiversity and heterogeneity of the cropped area are currently not well understood. However, there is some evidence that increasing this component of farmland heterogeneity can benefit biodiversity (Lindsay et al., 2013; Mitchell et al., 2014; Fahrig et al., 2015; Novotný et al., 2015). This suggests a potential conservation strategy to support farmland biodiversity without taking land out of crop production.

Heterogeneity of the cropped area of the landscape can be increased either by diversifying the crop types grown (higher compositional heterogeneity) or by growing them in a more complex spatial pattern (higher configurational heterogeneity) (Fahrig et al., 2011). Landscapes with higher crop diversity can support higher levels of biodiversity (Lindsay et al., 2013; Novotný et al., 2015) because different crops can provide resources for different species (Westphal et al., 2003; Le Féon et al., 2010; Novotný et al., 2015).

Fahrig et al. (2015) found consistent, positive relationships between farmland configurational heterogeneity (measured as lower mean crop field size) and diversity of birds, plants, and five different arthropod groups in agricultural landscapes. They hypothesized that species benefit from easy access to field boundary habitats in landscapes with small crop fields. Similarly, Mitchell et al. (2014) reported a decrease in arthropod diversity with increasing field widths in agricultural landscapes. Landscapes with high configurational heterogeneity may also facilitate animal movement, as field edges can be used as movement corridors by some species (Joyce et al., 1999; Holzschuh et al., 2009).

Amphibians are recognized as the most threatened class of vertebrates on the planet (Stuart et al., 2004), largely resulting from habitat loss due to agriculture (Gallant et al., 2007). Habitat degradation from agricultural activities further compromises amphibian populations. For example, agrichemical exposure can cause lethal and sub-lethal toxic effects such as endocrine disruption, immunosuppression, behaviour modification, and growth and developmental abnormalities (Bridges, 1999; Hayes et al., 2002; Howe et al., 2004; Relyea, 2005; Mann et al., 2009; Christin et al., 2013). Despite the negative effects of agriculture, some species have been found to be positively associated with high intensity crop cover (Koumaris and Fahrig, 2016). Habitats within farmland are regularly used by amphibians (Harding, 1997; Ouellet et al., 1997; Harris et al., 1998; Bishop et al., 1999; Knutson et al., 2004; Koprivnikar et al., 2006; Gagné and Fahrig, 2007; McDaniel et al., 2008; Christin et al., 2013; Koumaris and Fahrig, 2016), and in some areas are considered critical for the persistence of local populations (Bishop et al., 1999; Knutson et al., 2004). Many amphibian species use shallow farm wetlands

such as ponds, drainage ditches, and flooded fields for breeding (Harding, 1997; Ouellet et al., 1997; Harris et al., 1998; Knutson et al., 2004; Koprivnikar et al., 2006; Gagné and Fahrig, 2007; McDaniel et al., 2008; Koumaris and Fahrig, 2016). Woodlots in farmland are important terrestrial habitat for the adult stages of many species (Weyrauch and Grubb, 2004; Boissinot et al., 2015). As well, some species forage in agricultural fields (Harding, 1997; Attademo, 2005; Peltzer et al., 2010).

Although it is clear that natural areas such as forest patches and wetlands within agricultural landscapes are important for farmland amphibians (Knutson et al., 1999; Kolozsvary and Swihart, 1999; Porej et al., 2004; da Silva et al., 2011), it is not known whether the cropped area of the landscape can be structured in a way that benefits them. Identifying cropped cover patterns that are positively related to amphibian diversity would provide options for maintaining and enhancing amphibian diversity in agricultural regions. These options would be particularly valuable in regions where most of the natural habitats have been lost, and in situations where taking farmland out of production for conservation is not feasible.

The goal of this study is to identity farmland patterns that support amphibian diversity in agricultural landscapes. The specific purpose is to determine if anuran (frog and toad) species richness and abundance are associated with compositional and configurational heterogeneity of the cultivated areas in agricultural landscapes (hereafter 'farmland'). We hypothesized that both compositional and configurational heterogeneity of farmland should benefit anurans. A farmland with a high diversity of crop types should provide resources for different prey arthropod species (Westphal et al., 2003; Langellotto and Denno 2004; Le Féon et al., 2010; Novotný et al., 2015) at different times throughout

the growing season, thus providing a more temporally stable prey resource for anurans than a farmland with low crop diversity. In addition, farmlands with smaller crop fields should contain more anurans, due to the benefits of field edges. Edges often have higher arthropod abundance than crop fields (Molina et al., 2014) and therefore may serve as important anuran foraging habitat. We also suspect that field edges could facilitate amphibian movement through agricultural landscapes, as anurans have been shown to use riparian buffers along streams in farmland (Maisonneuve and Rioux, 2001; Maritz and Alexander, 2007). We therefore predicted higher anuran richness and abundance in farmlands with higher crop compositional and configurational heterogeneity.

We tested this prediction in a multi-landscape study in eastern Ontario, Canada. We surveyed anuran richness and abundance in each landscape. We measured farmland compositional heterogeneity as the Shannon diversity of crop types in a landscape and farmland configurational heterogeneity as the mean crop field size in a landscape.

2.3 Methods

Study sites

We selected 34 1-km radius agricultural landscapes, each centred on an anuran survey point, in Eastern Ontario, Canada, across an area of approximately 5 000 km^2 within the St. Lawrence River lowlands (Figure 2.1). Approximately 47% of this region is farmed, characterized by row crops (primarily corn, soybean, forage crops, and cereal grains), and pasture lands (EOWC, 2007; OMAFRA, 2011). Interspersed with farmland are patches of forest, wetlands, and some urban cover.

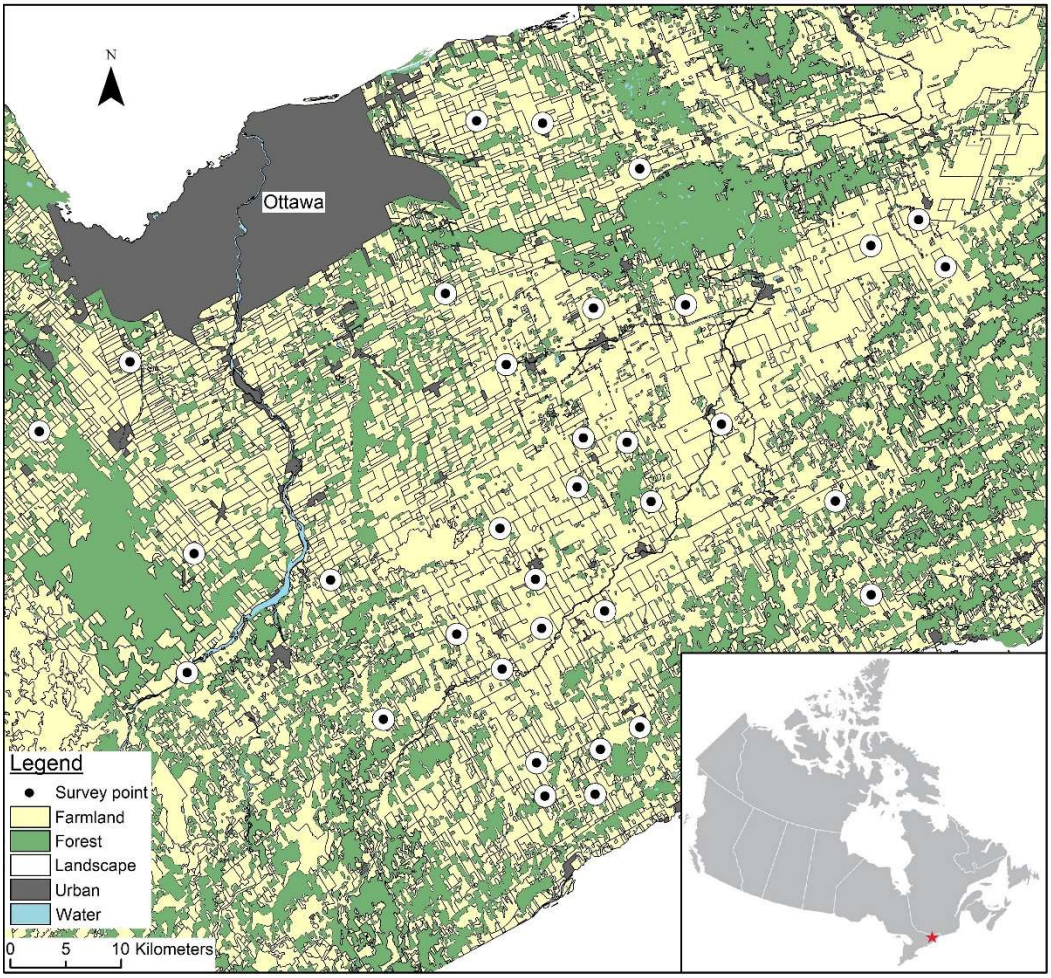

Figure 2.1. Thirty-four 1-km radius agricultural landscapes centered on anuran call survey points in eastern Ontario, Canada. Survey points are shown as black dots within the white circular landscapes.

We chose 1-km as the landscape size because this is considered a reasonable size to represent the average dispersal and migration movements for amphibians (Guerry and Hunter, 2002; Wagner et al., 2014), and landscape variables have been shown to affect anuran occupancy and diversity at this scale in agriculture-dominated regions (Vos and Stumpel, 1996; Knutson et al., 1999; Guerry and Hunter, 2002; Van Buskirk, 2005). We followed a methodological framework proposed by Fahrig et al. (2011) to select agricultural landscapes that represent gradients in compositional and configurational farmland heterogeneity. Preliminary 3 km x 3 km agricultural landscapes (> 45% cropland) were identified by Pasher et al. (2013) based on a land cover map of crop types originating from Landsat-5 satellite images from 2007. These were selected to (i) represent gradients in farmland heterogeneity, as measured by crop diversity and mean field size; (ii) ensure spatial independence (minimum distance of 3.5 km apart); (iii) minimize cross-landscape correlation between crop diversity and mean field size; and (iv) avoid regional trends in the landscape variables. Within each of a subset (n = 34) of the landscapes selected by Pasher et al. (2013), we located a single anuran survey point. Survey points were located at appropriate anuran breeding sites to standardize survey protocols and ensure there was a minimum amount of additional breeding habitat in the landscape. We chose to survey next to drainage ditches, as these represented the most common type of aquatic habitat in our study area, and have been reported to support amphibian breeding (de Solla et al., 2002). We adjusted the landscape boundaries to the circular areas (1 km radius) around each survey point. Within each 1-km radius landscape, we delineated field boundaries and crop types in each field during our study

year (2012), using aerial photographs taken in 2012 combined with ground surveys (see also Fahrig et al., 2015) (*e.g.* Figure 2.2).

Anuran surveys

We conducted night anuran auditory surveys for relative abundance of each species at each survey point, following standard anuran auditory survey protocols (Shirose et al., 1997; Pope et al., 2000; Crouch and Paton, 2002; TRCA, 2011). We surveyed each point four times on nights from 21 March to 4 June 2012, allowing for coverage of all expected species' breeding seasons. We surveyed points in a randomized order, with an average of 18 ± 4.02 (SD) days between surveys for a given point. We conducted surveys on warm nights, without heavy rain and with low wind (Beaufort wind scale ≤ 3). Minimum air temperatures for surveys were 5°C in March and April, and 10°C in May and June (modified from Shirose et al., 1997). We terminated surveying on a given night if the air temperature fell below these thresholds, or if high wind or heavy rain began (as suggested by TRCA, 2011). We surveyed an average of 4 to 5 points each evening between 30 minutes past sunset and 1:00 am. Surveys consisted of listening for anuran calls for 10 minutes at each point. It has been demonstrated that 3 to 5 minutes is adequate to hear most species (Shirose et al., 1997; Crouch and Paton, 2002). We recorded all species heard calling, irrespective of distance from the survey point. We assigned choruses an abundance rank based on the following classes: 0 (no calls), 1 (distinct calls of separate individuals), 2 (some calls overlap but most individuals can still be identified), 3 (mostly overlapping calls and continuous calling), as in Pope et al. (2000).

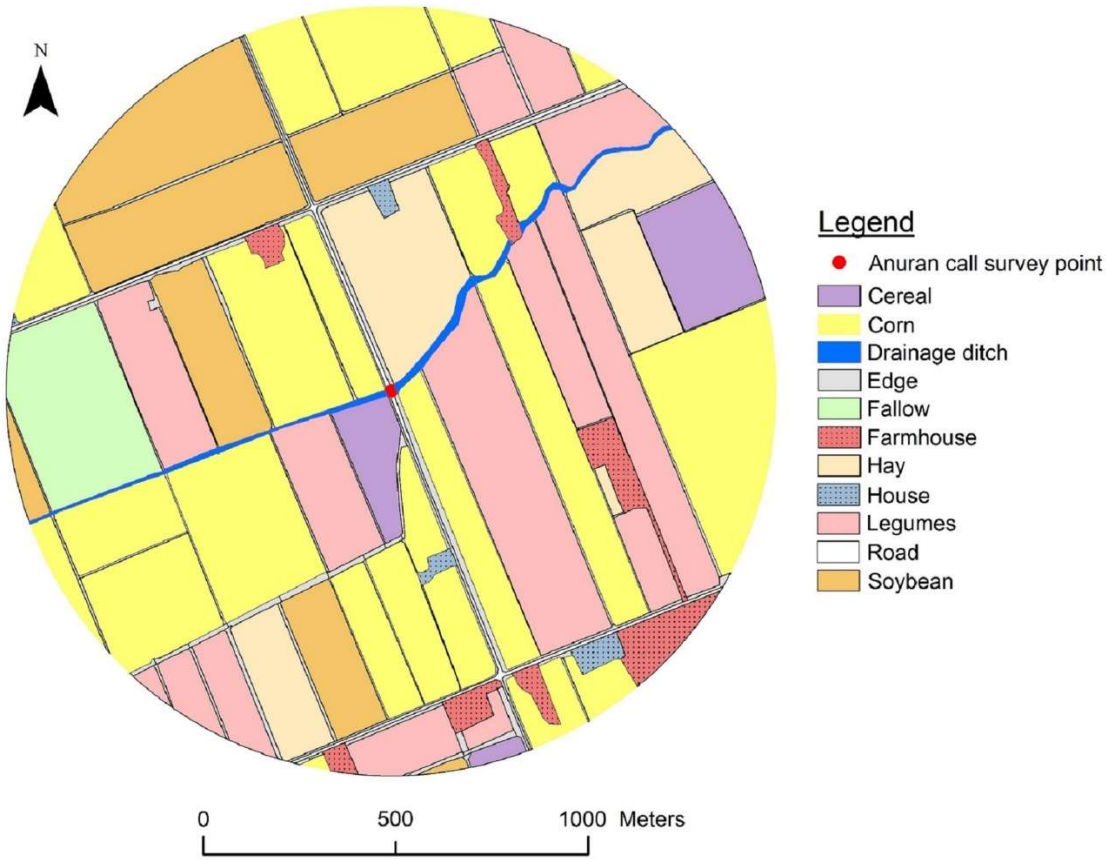

Figure 2.2. Example land cover map of one of the 34 1-km radius agricultural landscapes in eastern Ontario, Canada, showing the anuran call survey point and detailed land cover information determined through digitization of aerial photographs and validation by field crews.

Landscape Variables

Our two primary landscape variables were farmland compositional and configurational heterogeneity. We measured compositional heterogeneity as the Shannon diversity of crop types in each landscape. The crop Shannon diversity index accounts for both the number of distinct types present (crops, in this case) and proportions (evenness) of each. The proportional cover of each crop type relative to the total crop area is calculated, and then multiplied by the natural logarithm of this proportion. The resulting product is summed across crop types, and multiplied by -1 (Stiling, 2012). Crop types encountered in our landscapes were apple, canola, cereal, corn, hay, legumes, mixed vegetables, soybean, and sod. In addition to these we also included pasture and summer fallow as crop types in the Shannon diversity calculation because the pastures are often seeded and summer fallow is part of a crop rotation cycle. We measured farmland configurational heterogeneity as the mean crop field size in each landscape, where a smaller mean indicates higher configurational heterogeneity. We chose to use mean crop field size as our crop field configuration measure because altering field size is a potential management option for landowners.

In addition to these primary variables, we included two potential confounding variables, which we had not been able to control for during site selection, as covariates in statistical models: the area of forest cover and the area of soybean cover in landscapes. As forest is important habitat for anurans in agriculture-dominated landscapes (Hecnar and M'Closkey, 1998; Knutson et al., 1999; Guerry and Hunter, 2002; Porej et al., 2004), we predicted that anuran species richness and abundance would be positively associated with the proportion of forest cover in the landscape. Soybean cover has been shown to

entail a significantly lower dispersal cost for amphibians compared to other crops such as corn or grassland (Cosentino et al., 2011). Anuran species have also been reported to forage in soybean (Attademo et al., 2005). Based on this we predicted that anuran species richness and abundance would be positively associated with the proportion of soybean cover in farm landscapes.

Analyses

The response variables were: (i) species richness, i.e. the total number of anuran species detected at each survey point, (ii) an index of anuran total abundance at each point, i.e. the summed abundance ranks for all species, from all call surveys for the point (Pope et al., 2000), and (iii) presence/absence of each species at the point. The latter was limited to species with < 90% and > 10% to estimate reliable logistic regression coefficients. The predictor variables were crop Shannon diversity, mean crop field size (log transformed), proportion of forest cover (arcsine square-root transformed), and proportion of soybean cover for each landscape.

A multi-model inference approach using the second order Akaike's Information Criterion (AIC_c) (Burnham and Anderson, 2002) was used to calculate and compare standardized model-weighted mean coefficients to determine the direction and relative importance of the predictor variables on the response variables. For each response, the global model contained all four predictors. Since species richness and total abundance were count data, we ran these statistical models as generalized linear models (GLMs) with Poisson error structure and log-link function. Presence-absence models for individual species were run as GLMs with binomial error structure and logit-link function. We did not model species' presence-absence with occupancy modeling as our

sites were not closed to changes in occupancy during the sampling period (MacKenzie et al., 2006). Separation occurred when modeling the American toad data with the binomial GLM, so a GLM with a Bayesian function using a Cauchy prior distribution with a median of 0 and scale of 2.5 on each coefficient was used to produce stable estimates (Gelman et al., 2008).

Prior to model selection we assessed the fit of the global models by examining residual plots and calculating the adjusted R^2 (Burnham et al., 2011). The residuals did not appear heteroscedastic, and the adjusted R^2 values ranged from 0.11 to 0.43 (Appendix A, tables A.1 to A.9). There was no indication of overdispersion in the distribution of species richness; however, overdispersion was detected in the total anuran abundance model by the dispersion parameter of the global model (calculated by dividing the residual deviance by the residual degrees of freedom) (Mazerolle, 2006). We accounted for overdispersion by basing model selection on the quasi-AIC_c ($QAIC_c$). We adjusted the standard errors of the regression estimates by multiplying them by the square root of the dispersion parameter to reflect the additional variability in the data (Mazerolle, 2006; Ver Hoef and Boveng, 2007).

For each response variable, we performed model selection on a set of 16 candidate models, representing the global model which included all four landscape predictors, an intercept-only model, and all submodels derived from the global model. We chose to use the all-subset approach to model selection because we had biological hypotheses for each predictor, but did not know what particular combination of predictors (i.e. model) would best explain the data or what was the relative strength of each predictor. Each candidate model therefore represents a biologically relevant hypothesis.

To estimate the relative effects of the landscape variables we calculated the model-weighted mean standardized coefficients from all the models in the candidate set. All statistical analysis and data manipulations were performed in the R environment (R Core Team, 2015). We used the package AICcmodavg (Mazerolle, 2016) for model selection and averaging for all responses except American toad presence/absence. For this species we constructed the Bayesian models using the package arm (Gelman et al., 2015) and performed model selection and averaging using the package MuMIn (Barton, 2015).

2.4 Results

We detected eight anuran species across the 34 survey points. Spring peepers (*Pseudacris cruficer*) and American toads (*Anaxyrus americanus*) comprised the majority of observations; both species were detected at 88% of the points. The other 6 species were detected in the following proportion of points: gray tree frog (*Hyla versicolor*) (0.41), northern leopard frog (*Lithobates pipiens*) (0.26), green frog (*Lithobates clamitans*) (0.21), wood frog (*Lithobates sylvaticus*) (0.15), western chorus frog (*Pseudacris triseriata*) (0.12), and American bullfrog (*Lithobates catesbeianus*) (0.03). The highest species richness detected at a single point was 6 species, and average species richness across all points was 2.94 ± 1.41 (SD).

The amount of cropped cover in the 34 landscapes ranged from 55.8 to 90.9% (mean 72.5 ± 10.9 SD). The amount of forest cover ranged from 0 to 29.9% (mean 9 ± 7.9 SD), and the amount of soybean cover ranged from 0 to 47.5% (mean 20.3 ± 11.4). Mean crop field size in landscapes ranged from 2.04 to 15.9 ha (mean 5.43 ± 3.03 SD). Crop Shannon diversity ranged from 0.67 to 1.73 (mean 1.25 ± 0.34 SD). Pairwise correlations between the predictors are shown in Appendix B.1. Although the Pasher et

al. (2013) landscapes were selected with the goal of minimizing correlations between crop diversity and mean field size, the correlation between these two variables across the 34 circular landscapes in this study was -0.63. It is generally accepted that r = 0.7 is an appropriate maximum collinearity threshold for estimating independent effects of correlated variables (Dormann et al., 2013). We also calculated the Variance inflation factors (VIF) for each predictor in each model (Appendix B.2). The VIFs for all predictors were < 5, suggesting that collinearity was not severe enough to significantly impact the analysis (Zuur et al., 2007).

For both anuran species richness and abundance, model selection identified a top model and two additional models that were within ΔAICc/QAICc 2 units of the top model, indicating that the three models all have substantial support (Burnham and Anderson, 2002). Forest cover, crop diversity, and mean field size were within the top model set (ΔAICc < 2) for species richness (Appendix A, table A.1). The model selection results indicate that forest is the most important predictor, as it is present in every model in the top set (Appendix A, table A.1). The model-averaged coefficients and 95% confidence intervals identify forest as the only significant predictor for species richness (Figure 2.3). Species richness increased with forest cover (estimate 0.27 ± 0.11 SE, 95% CI [0.05, 0.49]). The associations between species richness and the farmland heterogeneity predictors were positive (Figure 2.3) though neither was statistically significant. Model-averaged coefficients suggest an increasing trend between species richness and crop diversity (estimate 0.19 ± 0.12 SE, 95% CI [-0.04, 0.42]) and a decreasing trend with mean field size (estimate -0.12 ± 0.16 SE, 95% CI [-0.43, 0.18]). Forest cover, mean field size, and crop diversity were within the top model set for total

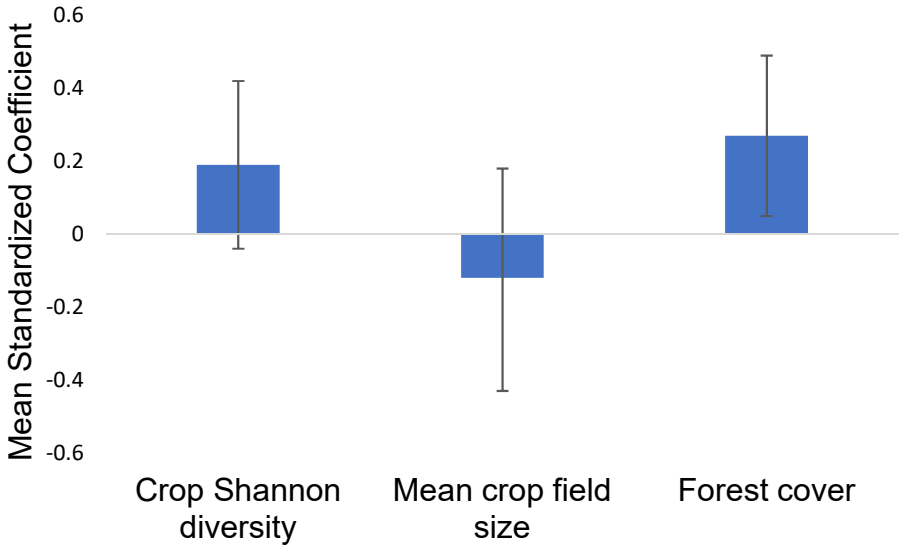

Figure 2.3. Model-weighted mean standardized coefficients and 95% confidence intervals showing the direction and relative magnitude of the effects of landscape variables (Shannon diversity of crop types, mean crop field size, proportion of forest cover) on anuran species richness in 1-km radius agricultural landscapes in eastern Ontario. All possible models were estimated, using generalized linear modelling. Each coefficient was model-averaged across estimates from all models containing that coefficient. Note that soybean cover is not shown as its model-averaged coefficient estimate was zero.

anuran abundance (Appendix A, table A.2). Forest cover, the strongest predictor, was positively related to total anuran abundance (estimate 0.37 ± 0.12 SE, 95% CI [0.13, 0.61]). Total abundance also decreased with increasing mean field size (estimate -0.29 ± 0.14 SE, 95% CI [-0.58, -0.01]), i.e. a significant positive association with configurational heterogeneity (Figure 2.4). The association between total abundance and crop diversity was positive (Figure 2.4), though not statistically significant (estimate 0.23 ± 0.13 SE, 95% CI [-0.03, 0.5]). The direction of effect of forest cover and both heterogeneity variables on species richness and abundance was consistent across all candidate models (positive for forest, positive for crop diversity, and negative for mean field size) which supports the authenticity of these relationships. The only significant effect of farmland heterogeneity on individual species responses was a positive relationship between American toad presence and crop Shannon diversity (Figure 2.5; estimate 1.93 ± 0.89 SE, 95% CI [0.11, 3.74]).

2.5 Discussion

While forest was the strongest predictor of anuran diversity in our agricultural landscapes, configurational heterogeneity of the cropped areas (farmland) was also positively related to anuran species abundance. Species richness and abundance increased with forest cover (Figures 2.3, 2.4), and abundance increased with decreasing mean field sizes (Figure 2.4). In addition, American toad presence was positively associated with compositional heterogeneity measured as crop diversity (Figure 2.5).

Our results are consistent with the prediction, based on several previous studies, that anuran species richness and total abundance are positively associated with forest cover in agricultural landscapes (Figures 2.3, 2.4). In addition, although the results for

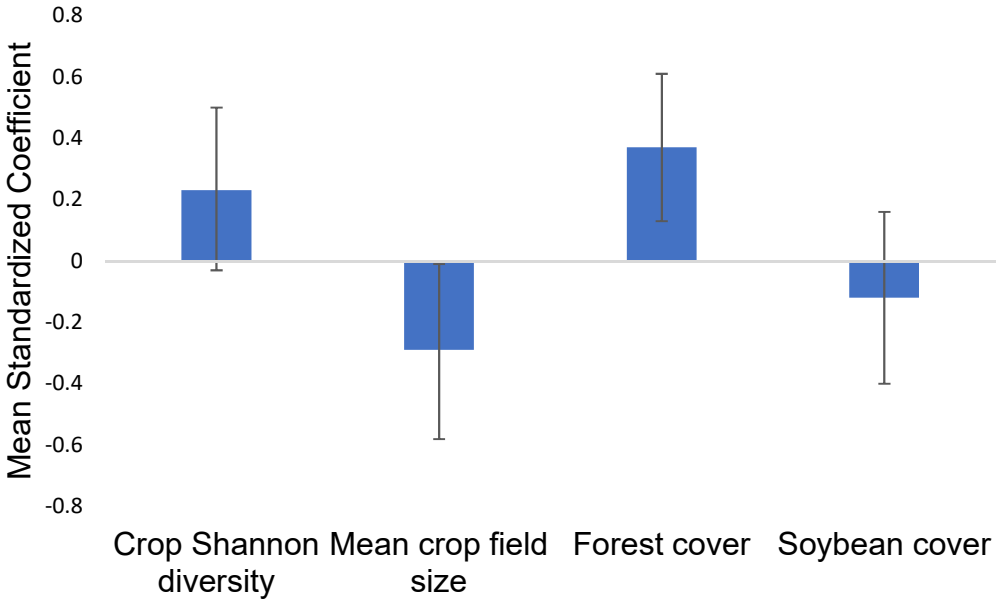

Figure 2.4. Model-weighted mean standardized coefficients and 95% confidence intervals showing the direction and relative magnitude of the effects of landscape variables (Shannon diversity of crop types, mean crop field size, proportion of forest cover, proportion of soybean cover) on total anuran abundance in 1-km radius agricultural landscapes in eastern Ontario. All possible models were estimated, using generalized linear modelling. Each coefficient was model-averaged across estimates from all models containing that coefficient.

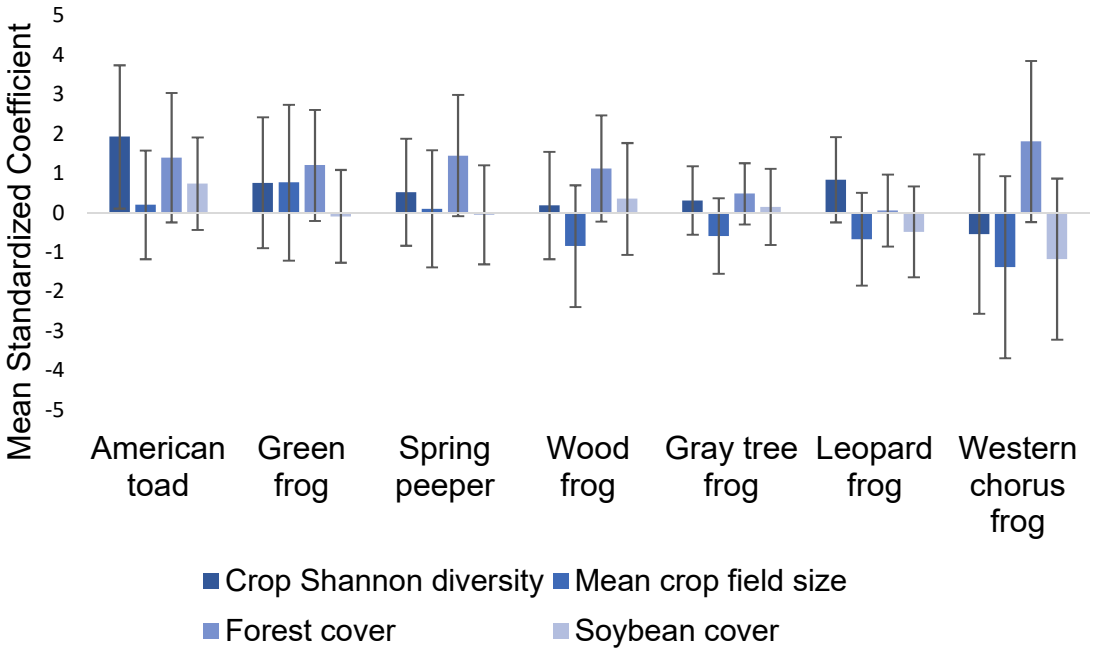

Figure 2.5. Model-weighted mean coefficients and 95% confidence intervals showing the direction and relative magnitude of the effects of landscape variables (Shannon diversity of crop types, mean crop field size, proportion of forest cover) on the presence/absence of 7 anuran species in 1-km radius agricultural landscapes in eastern Ontario. All possible models were estimated, using generalized linear modelling. Each coefficient was model-averaged across estimates from all models containing that coefficient.

individual species were not significant, the coefficients for forest cover were positive for all species (Figure 2.5), and forest was in the top model set for all species except the leopard frog (an open habitat species: Harding, 1997) (Appendix A, tables A.3 to A.9). This is consistent with many other studies showing that forest cover is an important predictor of anuran occurrence and/or diversity in agriculture-dominated landscapes (Hecnar and M'Closkey, 1998; Knutson et al., 1999; Guerry and Hunter, 2002; Porej et al., 2004). Forest is important terrestrial habitat for many amphibians (Harding, 1997; Guerry and Hunter, 2002), and many species use water bodies associated with wooded cover for breeding (Harding, 1997).

The negative response of anuran total abundance to mean field size is consistent with the hypothesis that farmlands with small crop fields should facilitate anuran movement, and also provide accessible refuge habitats. It has been suggested that linear structures in farmland such as hedgerows and ditches help maintain population connectivity for some anurans (Ficetola and Bernardi, 2004). Most anurans undergo seasonal migrations between different habitat types (Harding, 1997). Movement is more successful through humid cover types (Nowakowski et al., 2013) as anurans can quickly become desiccated in more open or barren areas such as some crop fields (Mazerolle and Desrochers, 2005; Youngquist and Boone, 2014). Farmlands with smaller fields have more field edge habitats. As these edges are generally more humid and provide more cover than crop fields (Forman and Baudry, 1984) farmlands with high configurational heterogeneity (low mean field size) likely provide refuge from dryer crop fields and facilitate movement through farmlands. Anurans may also benefit from higher prey

resources in agricultural landscapes with smaller mean field sizes, as crop field edges are higher in arthropod abundance than crop fields (Molina et al., 2014).

We can only speculate as to why the response of anuran abundance to mean field size was much stronger than the response of anuran richness to mean field size. The results indicate that increasing mean field size does not strongly reduce the likelihood of species presences, but does reduce their abundances. Given the strong effect of forest cover on anuran richness, we speculate that the presence/absence of a species in an agricultural landscape is primarily determined by the occurrence of that species' required habitats. While decreasing mean field size can enhance the abundances of the species present, species can occur even in landscapes with very large fields as long as the landscapes contain sufficient habitat. If this interpretation is correct, the results indicate that efforts to decrease mean field sizes may increase anuran abundances in farmlands, but do not obviate the need for maintaining or restoring natural habitats in these landscapes.

We are not aware of other studies investigating the relationship between mean crop field size and anuran richness and abundance. However, studies have reported negative relationships with crop field size (*i.e.* positive effects of farmland configurational heterogeneity) on birds (Lindsay et al., 2013; Fahrig et al., 2015), invertebrates (González-Estébanez et al., 2011; Mitchell et al., 2014; Fahrig et al., 2015), and plants (Gaba et al., 2010; Fahrig et al., 2015). Similarly, Flick et al. (2012) found positive effects of patch density on butterfly species richness in agricultural landscapes, and Novotný et al. (2015) found positive relationships between moth species richness and edge density in agricultural landscapes. Similar to our reasoning for anurans, Fahrig et al.

(2015) and Novotný et al. (2015) hypothesize that species benefit from smaller field sizes due to easier access to field edge habitats (*i.e.* landscape complementation) which facilitate movement through the landscape.

We had predicted that higher crop diversity should provide a more temporally stable prey resource for anurans, which should result in higher anuran richness and abundance in farmlands with more diverse crops. Overall our results did not support this prediction, with one exception: American toad presence increased with increasing crop Shannon diversity (Figure 2.5). This could be because the American toad is the species in our region whose biology most closely fits our prediction. The American toad is known to be mobile (Kolozsvary and Swihart, 1999; Pitt et al., 2013) and to use a wide range of human-modified habitats, including agricultural fields (Harding, 1997; Koumaris and Fahrig, 2016), gardens, and suburban areas (Harding, 1997). Therefore, it is likely that the American toad can move around in an agricultural landscape to benefit from the prey resources provided by different crop types at different times during the growing season.

We are not aware of other studies investigating the relationship between crop diversity and anuran diversity. There are, however, studies that report positive effects of crop diversity on birds (Firbank et al., 2008; Lindsay et al., 2013) and invertebrates (Östman et al., 2001; Novotný et al., 2015). Both Firbank et al. (2008) and Lindsay et al. (2013) found higher bird diversity in agricultural landscapes with higher crop diversity. Firbank et al. (2008), however, did not control for mean field size in their study, which makes interpretation of their positive effect of crop diversity somewhat ambiguous. Novotný et al. (2015) report higher moth species richness in agriculture landscapes with

higher crop diversity, and Östman et al. (2001) report better body condition of carabid beetles from farms with higher crop diversity.

Our results are not consistent with the prediction that anuran species richness and abundance are positively associated with soybean cover in agricultural landscapes (Figures 2.3, 2.4). We had hypothesized that anurans benefit from soybean cover in agricultural landscapes, based on a study suggesting that the movement cost through soybean was similar to that for forest, and lower than for corn (Cosentino et al., 2011). A possible reason why we failed to find a positive effect of soybean is because it is associated with high pesticide use in Ontario (McGee et al., 2010). Over one million kilograms of glyphosate active ingredient was applied to soybean fields in Ontario in 2008 (McGee et al., 2010). Glyphosate-based herbicide formulations have been shown to be highly toxic to amphibians (Howe et al., 2004; Relyea, 2005). Any positive effects of reduced movement cost through soybean may be negated by higher pesticide exposure.

We acknowledge that, as ours is a correlational study, the relationships we found are not necessarily driven by the mechanisms we hypothesized. In particular, there may be unmeasured confounding factors that are the true drivers of the relationships. For example, if landscapes with larger fields also have higher agrichemical use, higher rates of tillage, or more frequent use of irrigation, then the positive effect of configurational heterogeneity on anuran abundance we observed may be indirectly caused by an associated increase in these practices. Amphibians are known to be negatively affected by agrichemical exposure (Mann et al., 2009), and tillage may negatively affect their food base (Brévault et al., 2007). We do not know if mean field size and agrichemical use are related in our study region, but we note that only low correlations were reported from a

European study (Geiger et al., 2010). We are not aware of studies correlating tillage or irrigation practices to farmland configuration.

It is important to acknowledge that the correlation between crop diversity and mean field size (-0.63) introduces uncertainty in the estimates of the coefficients. We used model-weighted mean standardized regression coefficients to estimate relative importance of the predictor variables because they have been shown to provide unbiased estimates of relationships even in the presence of collinearity (Smith et al., 2009). In addition, the correlation coefficients and VIFs (Appendix B) are below standard cut-off thresholds for critical collinearity levels (Zuur et al., 2007; Dormann et al., 2013). Nevertheless, the fact that they are correlated means that conclusions about their relative effects should be taken with caution.

Our results cannot be used to support either of the major farmland conservation strategies, land sharing or land sparing, as we did not include estimates of agricultural yield for our landscapes. Land sharing involves reducing farming intensity, often associated with smaller field sizes, to support biodiversity. While land sharing does not require a loss of production area *per se*, reduced farming intensity is typically associated with reduced yields and therefore may require more farmed area (and less natural area) to meet production targets (Green et al., 2005; Baudron and Giller, 2014). In contrast, land sparing involves removing or reserving land from production while simultaneously maximizing yield output on the remaining farmlands (Green et al., 2005; Baudron and Giller, 2014). To determine which strategy is most appropriate in a given situation, both the biological responses to yield per unit area and production goals need to be modeled (as in Green et al., 2005).

What are the implications of our results for management of agricultural landscapes for anuran conservation? We found that anuran richness and abundance were most strongly related to forest cover, suggesting that preserving and restoring forest in agricultural landscapes is critical for maintaining or increasing farmland anuran richness and abundance. Forest represents habitat during at least part of the year for many of our species, so without at least some forest in a landscape, these species will be absent or in very low numbers. However, setting aside land for forest in an agricultural landscape means loss of land for crop production. Our results suggest that at least some benefit to anuran conservation can be obtained by altering crop field patterns in ways that increase configurational heterogeneity by reducing crop field sizes. We acknowledge that increasing configurational heterogeneity may present challenges and entail costs to farmers. For example, it may require investments in different farm equipment as machinery is often designed for large fields. However, alterations of the landscape pattern likely represent a more feasible conservation initiative for farmers to implement than removing land from production. Based on our results we suggest development of agri-environmental policies that maintain or enhance forest cover where possible, and that promote reduction of crop field sizes, to maintain and enhance anuran diversity and abundance.

Chapter 3 – Life in the slow drain: landscape structure affects farm ditch water quality

3.1 Abstract

Agrichemical contamination is a major threat to aquatic ecosystems in farmland. There is a need to better understand the influence of the surrounding landscape on farm wetlands to recommend land management options that minimize water quality impacts from agricultural practices. We tested hypothesized relationships between landscape structure and multiple water quality measures in farm drainage ditches in a multi-landscape study in Eastern Ontario, Canada. We measured physicochemical water quality (levels of atrazine, glyphosate, neonicotinoid insecticides, inorganic nitrogen, and dissolved oxygen), and biological water quality indicators (aquatic macroinvertebrate richness, leaf litter decomposition, and *Ceriodaphnia dubia* population responses) in 27 farm ditches, and measured the amounts of forest cover and high-intensity crop cover (landscape composition), and field edge cover (landscape configuration) in 1-km radius landscapes surrounding each ditch sampling site. We used confirmatory path analysis to simultaneously model the direct and indirect relationships between the landscape predictors and water quality variables. Landscape composition measures were the strongest predictors of water quality: pesticides decreased as surrounding forest cover increased, and nitrogen increased with increasing amounts of high-intensity crop cover. Crop cover was also indirectly negatively related to macroinvertebrate richness via its effects on nitrogen and dissolved oxygen. We found no effects of landscape configuration on agrichemical levels, but there was some support for a positive relationship between macroinvertebrate richness and field edge cover. Our results indicate that aquatic

macroinvertebrate richness is strongly impacted by fertilizer use in our region, and that macroinvertebrate richness is a more sensitive biotic indicator of farmland water quality than leaf litter decomposition or *C. dubia* responses. We conclude that, in our region, landscape management to improve farmland water quality should focus primarily on landscape composition. Such management should aim to increase amounts of non-crop cover such as forest, and reduce amounts of crop cover with high agrichemical inputs.

3.2 Introduction

Agriculture is associated with many leading threats to fresh water systems and aquatic biodiversity (Potter et al., 2004; Allan and Castillo, 2007). Land conversion for farmland has resulted in extensive aquatic habitat loss and modification (Blann et al., 2009; DUC, 2010). Conventional farming practices impact surface water quality by promoting sedimentation, nutrient enrichment, and agrichemical contamination (Allan and Castillo, 2007). Impairments to water quality impact biological communities by altering physicochemical characteristics of habitats, such as dissolved oxygen levels, and inducing toxic effects on biota (Camargo and Alonso, 2006).

The water quality of remaining farmland water bodies is influenced by the amount of different surrounding land cover types (Johnson et al., 1997). In general, water quality is negatively correlated with agricultural land cover and positively related to more natural cover, such as forest (Declerck et al., 2006; Liu et al., 2012; Gonzales-Inca et al., 2015; Li et al., 2015). Crop cover in the surrounding landscape negatively affects water quality through its role as a source of agrichemicals (Allan and Castillo, 2007). In contrast, forest cover is often positively associated with water quality not only because it limits the total amount of agrichemical-source cover within a landscape, but also because forest

vegetation can reduce agrichemical loads in runoff through chemical uptake and transformation (Burken and Schnoor, 1998; Näsholm et al., 1998).

The water quality of farmland water bodies might also be influenced by the spatial arrangement of cover types in the surrounding landscape, i.e. landscape configuration. Increasing the complexity of the landscape pattern ('configurational heterogeneity') increases the amount of edge (Figure 3.1). Positive relationships have been found between total edge density in catchments and the water quality of rivers (Uuemaa et al., 2005, 2007) and lakes (Liu et al., 2012). Uuemaa et al. (2007) speculate that more complex landscape patterns retain more nutrients in runoff, thus reducing inputs to water bodies. We therefore expect agrichemical levels in farmland water bodies to decrease with increasing landscape configurational heterogeneity.

In addition to chemical measures of water quality, aquatic organisms are also frequently used as water quality indicators (Eagleson et al., 1990; Baldy et al., 2007). Biotic water quality indicators could be indirectly related to landscape structure through the effects of landscape composition and configuration on water chemistry. Some indicators could also be directly influenced by landscape composition and configuration. For example, forest cover can provide habitat and facilitate the dispersal of some adult aquatic insects (Jonsen and Taylor, 2000; Didham et al., 2012) and vegetated field edges can provide refuge habitat and serve as movement corridors between forest patches for some adult aquatic invertebrates (Nasci, 1982; Burel et al., 2004).

Understanding the effects of landscape structure on farmland water quality can inform landowners and policy makers of effective options to minimize agrichemical inputs to surface waters and support biodiversity. Options involving changes to landscape

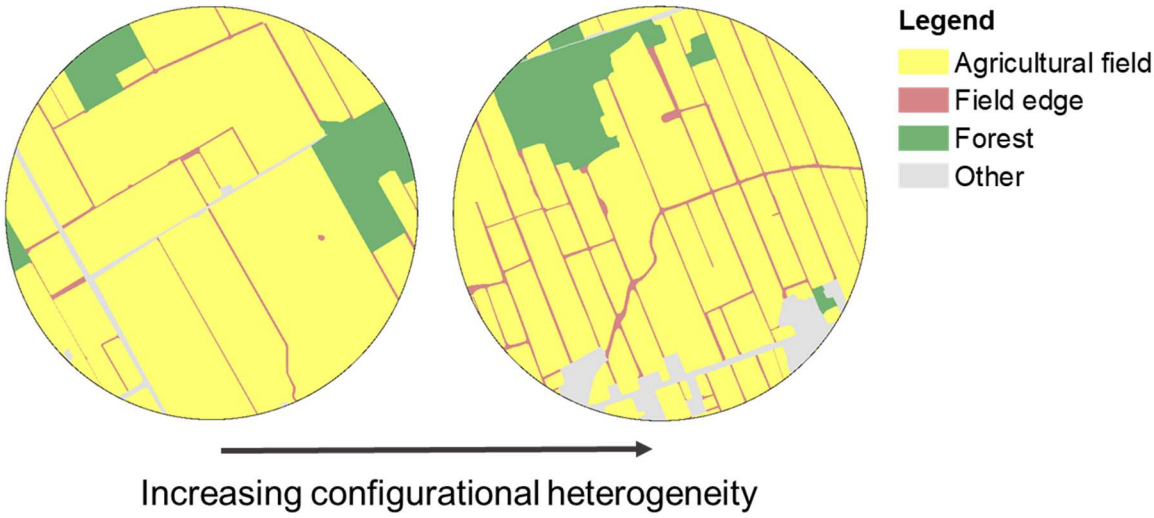

Figure 3.1. Two example agricultural landscapes used in this study, with similar amounts of forest and different amounts of field edge cover, to illustrate edge cover as a measure of landscape configuration. An increase in field edge cover indicates an increase in the complexity of the landscape spatial pattern (configurational heterogeneity).

configuration (rather than composition) would be particularly appealing because such options would not require taking land out of production. For example, increasing configurational heterogeneity by reducing mean crop field sizes has been suggested as an alternate conservation action to land retirement that can benefit biodiversity in agricultural landscapes (Fahrig et al., 2015; Collins and Fahrig, 2017; Monck-Whipp et al., 2018).

The goal of this study was to test hypothesized direct and indirect relationships between agricultural landscape structure (composition and configuration) and different measures of water quality in farmland drainage ditches, a common aquatic habitat in our study region. The predictions are shown in Figure 3.2. We tested these predictions in a multi-landscape study in Eastern Ontario, Canada, across an area of approximately 5 000 km^2 in the St. Lawrence watershed of the Mixedwood Plains ecozone. We measured physicochemical water quality (levels of atrazine, glyphosate, neonicotinoid insecticides, inorganic nitrogen, and dissolved oxygen), and biological water quality indicators (aquatic macroinvertebrate richness, leaf litter decomposition, and *Ceriodaphnia dubia* population responses) in farm drainage ditches, and measured the amounts of forest cover and high-intensity crop cover (landscape composition), and field edge cover (landscape configuration) in landscapes surrounding each ditch sampling site.

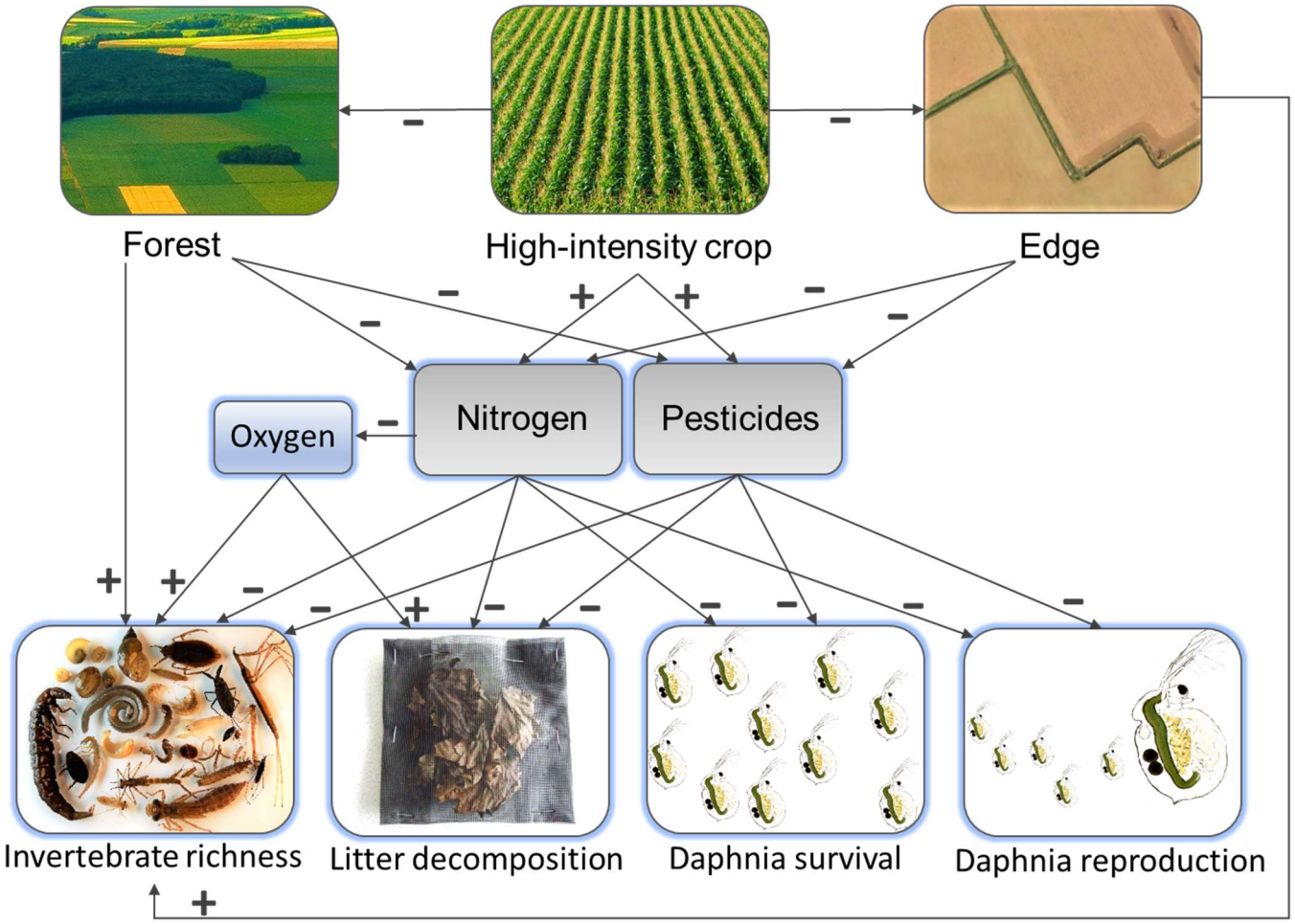
Forest
High-intensity crop
Edge
Oxygen
Nitrogen
Pesticides
Invertebrate richness
Litter decomposition
Daphnia survival
Daphnia reproduction

Figure 3.2. Predicted relationships between three landscape predictors (proportion of the landscape in forest, high-intensity crop, and field edge), physicochemical water quality variables (mean dissolved oxygen (mg/L), a linear combination of mean atrazine, mean glyphosate and summed mean clothianidin, imidacloprid, and thiamethoxam, and a linear combination of total ammonia and nitrate-nitrogen + nitrite-nitrogen), and biological water quality variables (aquatic macroinvertebrate family richness, leaf litter decomposition, and *Ceriodaphnia dubia* survival and reproduction in collected ditch water). Predicted relationships are represented as arrows originating from predictors and pointing to responses, and the predicted direction of effect is shown as + or –.

3.3 Methods

Study Sites

We selected 27 1-km radius agricultural landscapes, each centered on the middle of a 10-m survey transect in a farm drainage ditch, within Eastern Ontario, Canada (Figure 3.3). Approximately 47% of the study area is farmed, characterized by row crops (primarily corn, soybean, forages, and cereal grains), and pasture lands (EOWC, 2007; Mailvaganam, 2017). Interspersed with farmland are patches of forest (~31%), wetlands and open water (~7%), and some urban cover (~5%) (OMAFRA, 2010). The farmed portion of the region was once dominated by wetlands and wet forests, and has a flat topography and many areas of low-permeability soils (DUC, 2010; City of Ottawa, 2011). The advent of post-European settlement farming in the late 18th century necessitated extensive land drainage, resulting in a loss of approximately 70% of pre-European settlement wetlands (DUC, 2010; City of Ottawa, 2011). Networks of open-system constructed drains (drainage ditches) have been established in our region for at least 150 years (Irwin, 1989) and are now ubiquitous features across the region (Figure 3.3). Closed-system, subsurface drains (i.e. tile drains) are also widespread across our study region. Tile drains remove water from upper soil layers, thus lowering the water table and facilitating crop root growth (Blann et al., 2009; City of Ottawa, 2011). At least 40% of fields are tile-drained in our study region (EOWC, 2007; OMAFRA, 2016). While generally regarded as hydrologic infrastructures of agriculture, drainage ditches are also wetland habitats that support aquatic biota (Verdonschot et al., 2011) and provide important ecosystem services in farmland, such as flood and erosion control (Levavasseur et al., 2012), groundwater recharge (Dages et al., 2009), and water purification (Moore et

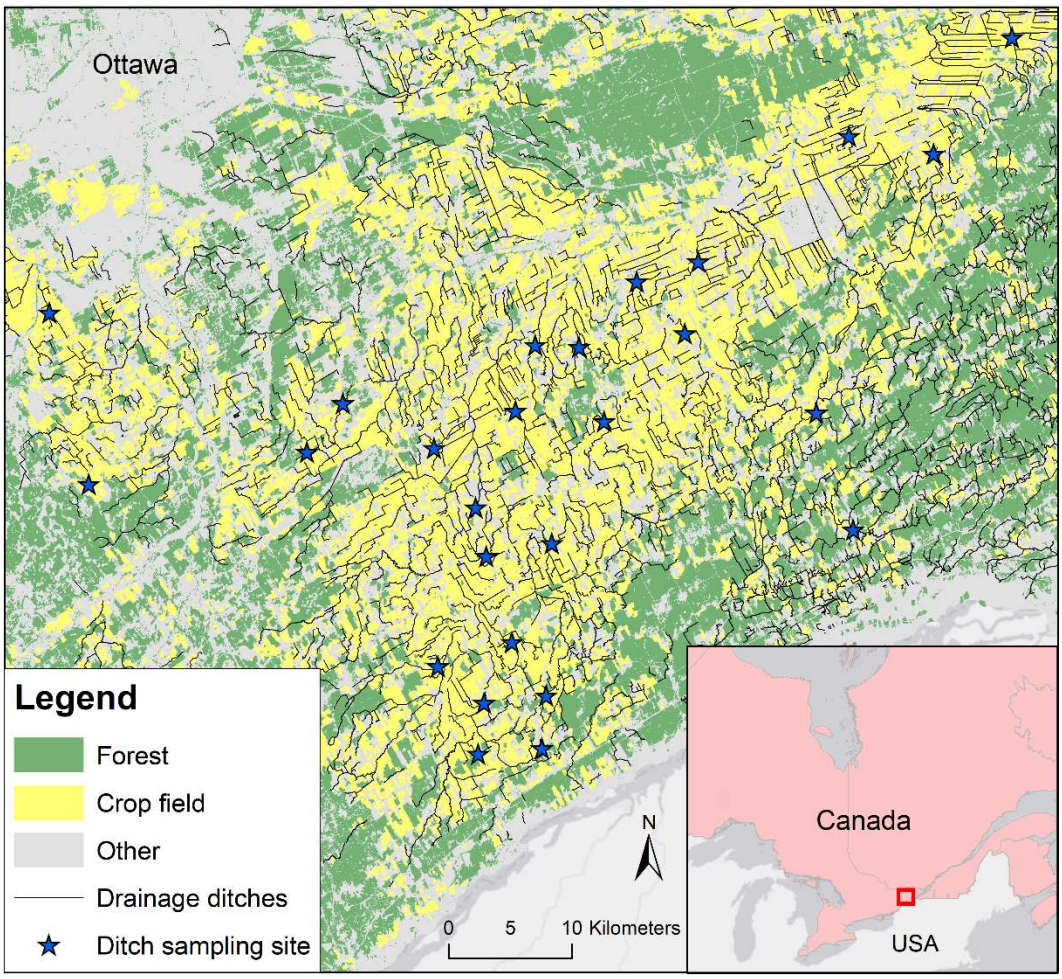

Figure 3.3. Agricultural drainage ditch sample sites (n = 27) in Eastern Ontario, Canada. Aquatic macroinvertebrates and water samples were collected from a 10-m long survey transect (blue stars) in each ditch during two collection periods in June and July 2014. The drainage ditch network (black lines) layer is from Ontario Ministry of Agriculture, Food and Rural Affairs (2016), and the forest and crop layers are from Agriculture and Agri-foods Canada (2014).

al., 2001). The 27 drainage ditches selected for this study are typical of the farmland ditches across the region (see Figure 3.4 for example ditch sites). Conversations with landowners involved in our study revealed that the ditches are decades old, with age estimates ranging from 25 to 80 years. Some landowners were unable to estimate the ages of their ditches, indicating that they are likely older than 80 years. We recorded ditch physical characteristics at three points (0, 5, and 10 m) along each ditch transect in June. Average June water depths ranged from 5 to 54 cm (mean 22 ± 11 SD). Average channel width ranged from 0.88 to 6.27 m (mean 1.9 ± 1.1 SD), and average channel bank height ranged from 0.93 to 3.13 m (mean 1.7 ± 0.6 SD).

We analysed relationships between ditch water quality measures and landscape structure within 1 km of the sampling site in each ditch. We defined landscapes as circular areas around sampling sites rather than ditch catchment areas because delineation of individual ditch catchments was not possible due to a lack of high resolution ground elevation data and the very flat nature of the region. We chose 1-km radius as the landscape size because previous work showed that high-intensity farming within this distance can influence the water quality of aquatic habitats (Koumaris and Fahrig, 2016), and because it encompasses the average dispersal and movement distances of many aquatic invertebrate terrestrial life stages (Kovats et al., 1996; Conrad et al., 1999).

Our ditch sites were located within a set of agricultural landscapes that were selected for a study investigating relationships between biodiversity and farmland heterogeneity, as measured by crop diversity and mean field size (Pasher et al., 2013; Fahrig et al., 2015). Landscape selection for this larger study aimed to sample spatially independent landscapes that represented the widest possible ranges in landscape

Figure 3.4. Examples of two of the 27 sampled farmland drainage ditches in agricultural landscapes of Eastern Ontario, Canada. Drainage ditches were sampled for water quality measures and these were related to surrounding landscape variables.

predictors while minimizing the cross-landscape correlation between them (Fahrig et al., 2011; Pasher et al., 2013). Landscapes that met these criteria were identified by Pasher et al. (2013) based on land cover maps (30 m pixels) created from Landsat-5 satellite images from 2007. Once the preliminary sample landscapes (3 km x 3 km) were selected, accurate maps were created in 2011 and 2012 using aerial photography (40-cm resolution) and ground surveys (Fahrig et al., 2015). We assumed there were no significant changes in the landscapes between 2011-12 and 2014. We selected a subset (n = 27) of these landscapes that represented gradients in agricultural intensity (measured as the amount of crop cover) and the amount of field edge cover and contained a drainage ditch. We also attempted to control for the proportion of road cover and water cover within landscapes. We established a 10-m sampling transect in the widest accessible section of each ditch, and adjusted the landscape boundaries to the circular areas (1 km radius) around the point in the center of each transect. The proportion of road cover in the final 27 landscapes ranged from 0.004 to 0.025 (mean 0.01 ± 0.005 SD) and open water/wetland cover ranged from 0 to 0.07 (mean 0.01 ± 0.02 SD).

Landscape Predictors

Our three landscape predictors were the proportion of the landscape in forest cover (square-root transformed), the proportion of the landscape in high-intensity agricultural cover (arcsine transformed), and the proportion of the landscape in edge cover, all in 1-km radius landscapes surrounding each ditch sampling site. High-intensity crop cover was the summed proportions of the landscape in the major crop types associated with high agrichemical inputs: corn, soy, and cereals (McGee et al., 2010). Edge cover was the proportion of the landscape covered in field edges. Field edges in our

study region are areas of unplanted land between adjacent crops, and are comprised of remnant, semi-natural strips of vegetation including varying proportions of grasses, forbs, shrubs, and trees. Our edge cover variable included all types of field edges, as we were unable to determine the composition of all edges in the landscapes due to access limitations during ground surveys. We considered the proportions of forest cover and high-intensity crop cover as measures of landscape composition. We considered edge cover as a measure of landscape configuration because the amount of field edge in a landscape is related to the spatial arrangement of agricultural cover via a negative relationship with mean field size (Figure 3.1). We standardized each of the three landscape predictors to a mean of 0 and SD of 1 prior to analysis. Predictor variable ranges, means, and standard deviations are shown in Table 3.1, and pairwise correlations are shown in Appendix C, Table C.1.

Water Quality Response Variables

Agrichemicals

We collected water samples twice from each 10 m ditch transect during two collection periods from 6 to 13 June and 7 to 15 July 2014 for pesticide analysis, and once during the second collection period in July for inorganic nitrogen analysis. Agrichemical levels in surface waters are highest this time of year from runoff inputs following planting and post-planting applications and from seed treatments (Thurman et al., 1992; Hladik et al., 2014). The first sampling period began following an extended period of rainfall (four days) to maximize detection of agrichemical pluses from post-planting runoff. Grab samples were collected from the center of each ditch transect and kept in coolers until returned to the laboratory. At each sampling site during both

collection periods we measured dissolved oxygen (DO) using a Horiba Scientific LAQUAact DO Meter OM-71, and we recorded pH and temperature using a Horiba Scientific LAQUAact pH meter.

Water samples were analyzed for pesticides at the National Wildlife Research Centre (NWRC) in Ottawa, Ontario, Canada, where high-performance liquid chromatography and tandem mass spectrometry were used to determine concentrations of atrazine, glyphosate, clothianidin, imidacloprid, thiamethoxam, and acetamiprid. Samples for atrazine and neonicotinoid analyses were held in 500 ml amber glass bottles and stored at 4 ºC, and samples for glyphosate analysis were held in 500 ml plastic bottles and stored at -40 ºC until analysis. The concentrations of the two herbicides were determined by methods developed by Laboratory Services at the National Wildlife Research Centre and the method used for neonicotinoid detection was adapted from Xie et al. (2011). Samples for all pesticide analyses were concentrated in duplicate using solid-phase extraction to achieve lower limits of detection prior to analysis. All analyses were performed on a high-performance liquid chromatograph (1200 Series, Agilent Technologies) with a tandem mass spectrometer (API 5000 Triple Quadrupole Mass Spectrometer and Turbo V^{TM} Ion Source, AB Sciex) with the ion source in positive polarity using multiple reaction monitoring. Details of all analytical methods and quality assurance are provided in Appendix D. The limits of detection for atrazine and glyphosate were 0.0004 µg/L and 0.025 µg/L, respectively. The limits of detection for the neonicotinoids were 0.00025 µg/L for clothianidin and imidacloprid, 0.0002 µg/L for thiamethoxam, and 0.0001 µg/L for acetamiprid. Acetamiprid was not detected in any of the samples. We summed the mean concentrations of clothianidin, imidacloprid, and

thiamethoxam for a given site to obtain a total neonicotinoid concentration (as in Main et al., 2015), as these compounds have similar structure and predicted additive toxicity (Morrissey et al., 2015).

Water samples were analyzed for inorganic nitrogen at Caduceon Environmental Laboratories in Ottawa, following standard methods of the Environment Laboratory Services Branch of the Ontario Ministry of the Environment. Samples were stored in plastic bottles at 4 ºC until analysis. Total ammonia (NH_3–N and NH_4–N) was determined by Colourimetry (method E3364), and nitrate-nitrogen (NO_3–N) and nitrite-nitrogen (NO_2–N) were determined by ion chromatography (method 4110 C) (Ontario Ministry of the Environment, 2010). Detection limits were 0.01 mg/L for total ammonia and 0.1 mg/L for nitrate-nitrogen and nitrite-nitrogen.

Biological Indicators

Aquatic Macroinvertebrate Family Richness

We sampled aquatic macroinvertebrates from each of the ditch transects during the two water collection periods in June and July, after the water sampling (above). We used a jab and sweep technique to sample all microhabitats, including the water column, substrate, aquatic vegetation, and woody debris, in each transect (modified from U.S. EPA, 1997; and Barbour et al., 1999). We collected invertebrates using a modified dip net measuring 56 cm x 48 cm with 5 cm net depth and 1 mm mesh size, by jabbing into dense aquatic vegetation and woody debris, and then sweeping through the water column with approximately 1-m long sweeps, 10 times per transect. This also included sampling substrate habitat by lightly sweeping the net along the ditch bottom. Invertebrates were

preserved in 70% ethanol on site. We identified specimens to family in the laboratory and used the total number of families per site from both collection periods as aquatic macroinvertebrate family richness.

Leaf Litter Decomposition Experiments

We determined the weight loss of leaf litter enclosed in mesh bags and exposed to water in ditches for one month as an assessment of decomposition rate in each ditch, where faster rates of decomposition generally indicate healthier aquatic systems (Mathews and Kowalczewski, 1969; Gessner and Chauvet, 2002). During the first water collection period (see above) in June, we submerged a 20 cm by 20 cm, 1-mm mesh bag containing 3 g of air-dried (for 2 weeks) mixed deciduous leaves, in each of the 27 ditches. The bags were placed in spots where they would remain fully submerged and minimally disturbed. We retrieved bags after one month during the second collection period in July. We kept bags chilled to prevent further decomposition during transit to the laboratory where they were immediately processed. In the laboratory, we removed all the leaf litter from each bag and gently rinsed the leaf litter with distilled water to remove sediment before oven drying (60 ºC for 24 h; as in Fernández et al., 2016) to obtain dry weight. We used the proportion of initial weight lost as a measure of decomposition rate. The bags from two sites were not retrieved and these sites were therefore excluded from statistical models that involved decomposition (see section 2.4 below).

Ceriodaphnia dubia Bioassays

We measured population responses of an indicator species, the daphnia *Ceriodaphnia dubia*, to ditch water exposure in 27 laboratory bioassays, to determine if

survival or reproduction responded to agrichemicals in ditch water samples. For this we used water collected during the June sampling period. Bioassays followed whole effluent chronic toxicity test guidelines for *C. dubia* (Environment Canada, 2007). We obtained a stock culture of *C. dubia* from Environment Canada at the beginning of May 2014, and established and maintained cultures to produce *C. dubia* neonates for testing (Environment Canada, 2007). We kept all cultures and experiments in a walk-in environmental chamber at 25 ± 1 ºC, 40% humidity, and a daily photoperiod of 16 ± 1h light and 8 ± 1h dark. We gave daily feedings of cultured algae (*Pseudokirchneriella subcapitata*) and a standard mixture of yeast, alfalfa, and trout chow (Environment Canada, 2007). Each eight-day bioassay consisted of recording *C. dubia* survival and reproduction in 10 replicates of 15 ml of undiluted ditch water using a single *C. dubia* female neonate < 24 h old in each replicate. The 10 neonates selected for each bioassay were within 12 h of the same age and reared in uncontaminated water that best matched the water hardness level (moderate vs hard) of each tested ditch water sample. We renewed test solutions daily and during this time recorded any mortalities and the number of neonates produced per test individual. Our survival variable was the total number of test individuals (of 10) alive at the end of each bioassay. Our reproduction variable was calculated for each 8-day bioassay as the average number of offspring produced per live individual ($n \leq 10$), per day.

Statistical Analyses

To avoid over-fitting our statistical models, we reduced the number of agrichemical variables using principal components analysis (PCA), to linearly combine correlated variables. We performed two PCAs, one to produce a composite variable for

pesticides (combining mean atrazine, mean glyphosate, and mean sum of neonicotinoids) and the other to produce a composite variable for inorganic nitrogen (combining total ammonia and the sum of nitrate-nitrogen and nitrite-nitrogen). All agrichemical variables were square-root transformed to normalize distributions and homogenize variances prior to statistical analyses. Agrichemical levels reported as less than the limit of detection (LOD) were set to 0, as in Balestrini et al. (2016).

We tested our predictions (Figure 3.2) using confirmatory path analysis (Shipley, 2000, 2009) because it easily accommodates small to moderate sample sizes and count data. This allowed us to simultaneously model the direct and indirect relationships between the landscape predictors and our water quality measures. Forest and crop cover, and field edge amount, were predictor variables in our model; however, forest cover and edge amount were also defined as responses to crop cover because the amount of a landscape in forest cover and edge amount is constrained by the amount of crop cover. As described above, seven water quality response variables were included in the path model: an index of pesticide amount; an index of inorganic nitrogen amount; mean dissolved oxygen (mg/L); aquatic macroinvertebrate family richness; leaf litter decomposition amount; *C. dubia* survival; and *C. dubia* reproduction.

We used the directional separation test to evaluate the correlational structure of our hypothesized path model before parameterizing individual paths between variables (Shipley, 2000; Gonzalez-Voyer and von Hardenberg, 2014). The directional separation test involved testing each implied independency (i.e. every pair of variables predicted to lack a direct relationship) in the path model in a linear or generalized linear model to determine the probability that each pair was statistically independent (conditional on the

hypothesized predictors in Figure 3.2). The correlational structure of the full path model was then tested by combining all the probabilities using Fisher's *C* statistic:

$$C = -2\sum_{i=1}^{k}(\ln(pi))$$

and comparing the resulting *C* value to a chi-square distribution with 2*k* degrees of freedom, where *k* = the total number of independencies (Shipley, 2000).

After determining that the correlational structure of the path model fit the data, we obtained individual path coefficients for the hypothesized paths by fitting a series of linear or generalized linear models for each response variable in the path model (Figure 3.2). Aquatic macroinvertebrate family richness and number of surviving *C. dubia* were modeled as count data using generalized linear models with a Poisson distribution and log-link function, and the other 7 response variables were modeled using linear models.

We further used a model selection approach (Burnham and Anderson, 2002) using Akaike's information criterion corrected for small sample sizes (AIC_c) to evaluate the statistical support for individual hypothesized paths in the full path model (Figure 3.2). For each of the nine response variables we created a set of candidate models comprised of a global model (i.e. the model containing all predictors hypothesized to influence the response; Figure 3.2), an intercept-only (null) model, and all submodels derived from the global model. We considered a predictor to have strong support if it was in the top (lowest AIC_c) model. We considered a predictor to have weak support if it was included in the top model set (within 2 AIC_c units from the top model) but not included in the top model. In addition, if the null model was not the top model but was within in the top

model set we considered predictors in the top model to have weak support. All analyses were conducted in R (R core team, 2018). Model selection was performed using the package AICcmodavg (Mazerolle, 2017). Prior to model selection we assessed the fit of all global models by examining residual plots. Residuals did not appear to violate assumptions of error distribution or homogeneity. We assessed the potential influence of multicollinearity by examining the Pearson correlation coefficients between all pairs of predictors and calculating the Variance inflation factors (VIF) for each predictor in each model. Pairwise correlations between predictor variables in the path model were all < 0.6 (Appendix C, Table C.1) and the variance inflation factors for each predictor in each model were all < 3 (Appendix C, Table C.2). These values are below accepted maximum collinearity thresholds for estimating independent effects of predictors (r < 0.7; Dormann et al., 2013, and VIF < 5; Zuur et al., 2007).

3.4 Results

Descriptive statistics for the ditch water quality and land cover variables measured from the 27 landscapes are in Table 3.1, and macroinvertebrate families identified from the sites are listed in Appendix E. Nitrate-nitrogen was detected in water collected from 26 sites during the July collection period, and samples from eight sites had concentrations that exceeded the Canadian water quality guidelines long-term exposure maximum level for the protection of aquatic life (3.0 mg/L; CCME, 2012). Atrazine was detected in water collected from every site, and the highest concentration in a sample from the June collection period exceeded the maximum level of the water quality guidelines for the protection of aquatic life (1.8 µg/L; CCME, 1999). Glyphosate was detected in water collected from 19 sites and neonicotinoids were detected in water

Table 3.1. Ranges, means, and standard deviations of variables used in the path model of the predicted relationships between drainage ditch water quality responses and landscape predictors (Figure 3.2), and the number of ditch sites in which agrichemicals were detected. Pesticides, dissolved oxygen, and aquatic macroinvertebrate richness variables were measured twice from each of 27 ditches. Inorganic nitrogen and daphnia (*C. dubia*) survival and reproduction variables were measured once in water from the 27 ditches, and leaf litter decomposition rate was measured once in 25 ditches. Land cover variables were measured in 1-km radius landscapes surrounding each ditch sampling site. "LOD" = limit of detection.

Variable	Range	Mean	SD	No. sites detected
Physicochemical water quality responses [a]				
Pesticides				27
Atrazine (µg/L)	0.005 - 2.76	0.17	0.44	27
Glyphosate (µg/L)	< LOD - 6.18	0.38	1.05	19
Total Neonicotinoids (µg/L)	< LOD - 0.61	0.04	0.09	26
Clothianidin (µg/L)	< LOD - 0.42	0.02	0.06	26
Imidacloprid (µg/L)	< LOD - 0.01	0.001	0.002	17
Thiamethoxam (µg/L)	< LOD - 0.23	0.02	0.04	26
Inorganic Nitrogen				26
Total ammonia (mg/L)	< LOD - 0.37	0.04	0.08	17

Nitrate-N (mg/L)	< LOD - 9	2.62	2.75	26
Nitrite-N (mg/L)	< LOD - 0.3	0.02	0.07	2
Dissolved oxygen (mg/L)	0.23 - 20	8.87	4.71	
Biological water quality responses				
Aquatic macroinvertebrate family richness (total no. families)	7 - 22	15.3	4.3	
Decomposition (proportion of initial weight lost)	0.19 - 0.93	0.50	0.21	
Daphnia survival (no. test individuals alive of 10)	5 - 9	6.6	1.2	
Daphnia reproduction (avg. mean no. neonates/individual/day)	1.95 - 5	3.17	0.81	
Landscape Predictors (% cover)				
Forest	0 - 34	9	9	
High-intensity crop	5 - 86	55	20	
Edge	2 - 6	4	1	

collected from 26 sites. Clothianidin and thiamethoxam were more commonly detected (each at 26 sites) than imidacloprid (17 sites). However, imidacloprid is the only neonicotinoid that has Canadian Environmental Water Quality guidelines documentation and recommendations. Two total neonicotinoid levels detected in the water samples exceeded the imidacloprid guideline for the protection of freshwater life (0.23 µg/L; CCME, 2007). We identified 54 families of aquatic macroinvertebrates representing 16 orders across the 27 ditches. The number of families detected per ditch ranged from 7 to 22 and mean number of families across ditches was 15. The gastropod family Physidae was the most common taxon, present in 25 sites (Appendix E).

The first component of the pesticide PCA accounted for 58% of the variation and was comprised of strong positive loadings (> 0.6) for all three pesticide variables (Appendix F, Table F.1). The first component of the PCA of the nitrogen variables accounted for 61.7% of the variation in the nitrogen data, with equal loadings of 0.78 for both total ammonia and nitrate-nitrogen + nitrite-nitrogen (Appendix F, Table F.2).

The C value calculated from the independency tests (Appendix G) was 45.9 with $2k = 48$ degrees of freedom, resulting in a null probability of 0.56. This indicates that the data fit the hypothesized patterns of independence implied by the model (Figure 3.2). Results of the tests of hypothesized paths are in Appendix H. Path coefficients and paths with statistical support are shown in Figure 3.5. Our analyses supported two of our hypothesized linkages between landscape composition and water quality, and one of our hypothesized linkages between landscape configuration and water quality (Figure 3.6): (i) a negative relationship between pesticide levels and the proportion of forest cover in the landscape (composition), (ii) a positive relationship between nitrogen levels and the

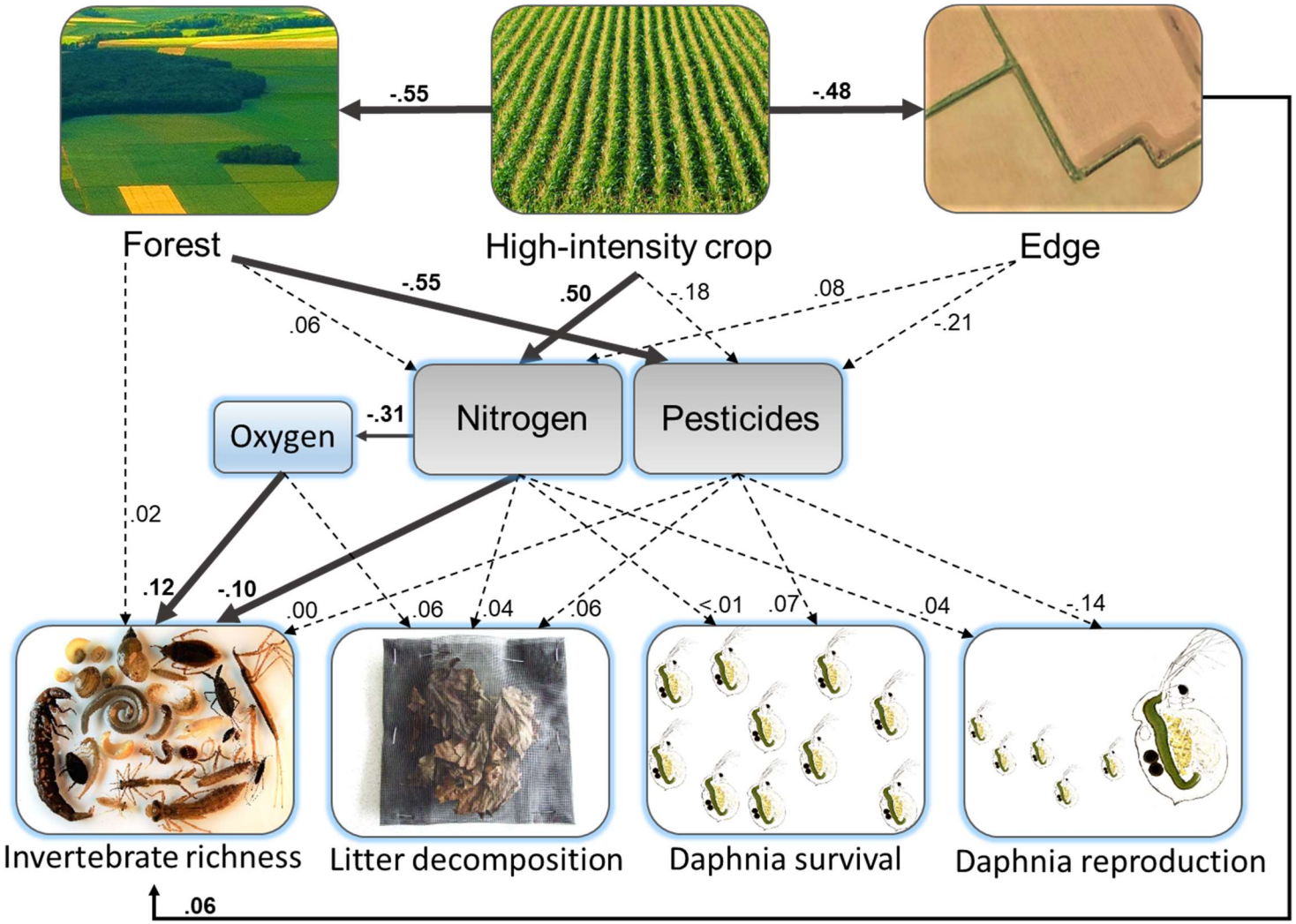
-.55
-.48
Forest
High-intensity crop
Edge
-.55
.50
-.18
.08
.06
-.21
Oxygen
-.31
Nitrogen
Pesticides
.02
.12
-.10
.00
.06
.04
.06
<.01
.07
.04
-.14
Invertebrate richness
Litter decomposition
Daphnia survival
Daphnia reproduction
.06

Figure 3.5. Observed relationships among landscape predictors, physicochemical water quality variables, and biological water quality responses in 27 drainage ditch transects. Landscape predictors (square-root transformed forest cover, arcsine transformed high-intensity crop cover, and edge cover), were measured in 1-km radius landscapes surrounding each transect. Results for all predicted paths from Figure 3.2 are shown. Paths with strong statistical support are represented by thick solid arrows, paths with weak support are represented by thin solid arrows, and paths without support are shown as dashed arrows (analytical results in Appendix H). Standardized path coefficients were derived from the global model for each response.

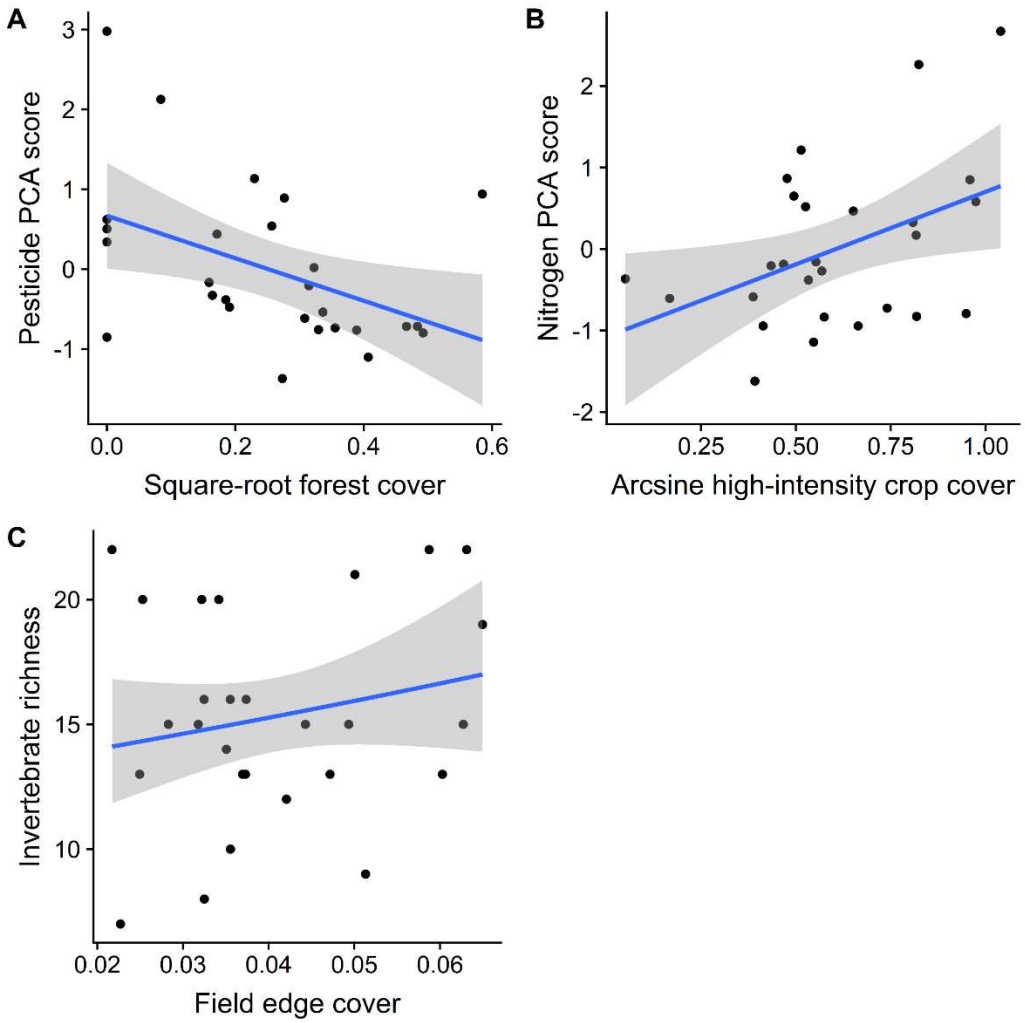

Figure 3.6. Relationships between landscape variables and water quality measures from 27 agricultural ditches. (a) Pesticides (a linear combination of square-root transformed mean atrazine, square-root transformed mean glyphosate, and square-root transformed summed mean clothianidin, imidacloprid, and thiamethoxam) vs. proportion of forest cover (square-root transformed) in the surrounding 1-km radius landscapes. (b) Inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen) vs. proportion of high-intensity crop cover

(arcsine transformed) in the surrounding 1-km radius landscapes. Shaded areas represent 95% confidence intervals. (c) Aquatic macroinvertebrate family richness vs. proportion of field edge cover in the surrounding 1-km radius landscapes.

proportion of high-intensity crop cover in the landscape (composition), and (iii) weak support for a positive relationship between macroinvertebrate richness and the proportion of edge cover in the landscape (configuration). Our analyses also supported three links among the water quality indices (Figure 3.7): (i) weak support for a negative relationship between dissolved oxygen and nitrogen levels, (ii) a negative relationship between macroinvertebrate richness and nitrogen levels, and (iii) a positive relationship between macroinvertebrate richness and dissolved oxygen.

3.5 Discussion

Our results confirm that the water quality of small farm wetlands is influenced by the surrounding landscape (Figure 3.5). Pesticide levels decreased as forest cover increased in the surrounding landscape, and inorganic nitrogen increased with increasing amounts of high-intensity crop cover (Figure 3.6). These results are consistent with previous studies that have examined relationships between water quality of water bodies and the surrounding landcover within circular buffers (Declerck et al., 2006; Koumaris and Fahrig, 2016) or within watersheds (Liu et al., 2012; Gonzales-Inca et al., 2015). We also found evidence that the amount of crop cover in the landscape indirectly decreases oxygen levels and aquatic macroinvertebrate richness, through increasing nitrogen levels in ditches (Figures 3.5 and 3.7). Together, the results are consistent with the hypothesis that forest cover reduces agrichemical loads in runoff through chemical uptake and transformation, while agricultural cover is a nonpoint source of agrichemicals to surface waters.

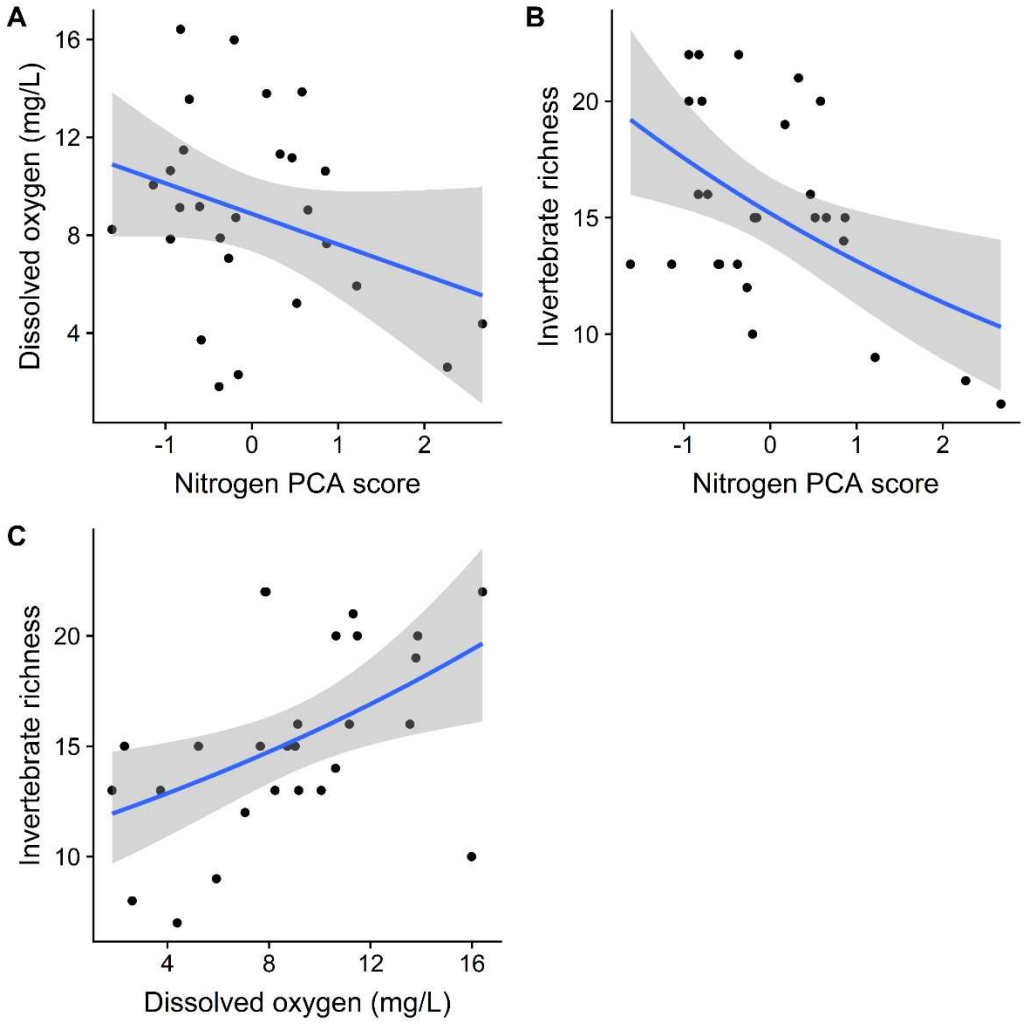

Figure 3.7. Relationships among water quality variables measured in 27 agricultural ditches. (a) mean dissolved oxygen (mg/L) vs. inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen). (b) Aquatic macroinvertebrate family richness vs. inorganic nitrogen. (c) Aquatic macroinvertebrate family richness vs. mean dissolved oxygen (mg/L). Shaded areas represent 95% confidence intervals.

We did not find support for the hypothesis that increasing landscape configurational heterogeneity, i.e. increasing field edge cover, reduces agrichemical inputs to farmland ditches (Figure 3.5). This is in contrast to studies that found positive relationships between water quality and edge density in the landscape (Uuemaa et al 2005; Uuemaa et al 2007; Liu et al 2012). However, some previous results are mixed (Lee et al., 2009; Gémesi et al., 2011; Li et al., 2015). We suggest that the lack of effect of edge cover on water quality in our study is likely due to the very flat topography of our region, combined with the widespread use of tile drainage in the crop fields. Tile drains can bypass subsurface water flows beneath strips of native vegetation, preventing the interception of water by plant roots and opportunities for filtration and uptake, and discharging directly into surface waters (Blann et al., 2009). This could negate potentially beneficial effects of field edges on water quality in farmland in our region. It is possible that edges have stronger positive effects on water quality in regions without tile drainage than regions with tile drainage. Testing this post-hoc hypothesis would require further research.

While landscape composition measures were the strongest predictors of water quality in ditches, we also found some support for an association between water quality and landscape configuration: aquatic macroinvertebrate family richness was positively related to field edge cover in the surrounding landscape (Figures 3.5 and 3.6). This result is in line with previous work suggesting that increasing configurational heterogeneity in agricultural landscapes can increase biodiversity (Holland and Fahrig, 2000; González-Estébanez et al., 2011; Flick et al., 2012; Fahrig et al., 2015; Novotný et al., 2015; Collins and Fahrig, 2017; Monck-Whipp et al., 2018). For example, the richness of some

terrestrial insect groups has been positively associated with edge density (Novotný et al. 2015), patch density (Flick et al., 2012), and the amount of woody borders (Holland and Fahrig, 2000) in agricultural landscapes, and insect diversity can be negatively related to mean crop field size (González-Estébanez et al., 2011; Fahrig et al., 2015). Our results suggest the same is true for aquatic insects in agricultural landscapes and is consistent with the hypothesis that field edges benefit some taxa by providing habitat and facilitating movement through the landscape.

Taken together, the lack of effect of configurational heterogeneity on agrichemical inputs and the positive effect of configurational heterogeneity on biodiversity suggest that different management guidelines will be needed for the provision of different ecosystem services in farmland. Services such as pollination and biological pest control, which are provided by terrestrial and semi-aquatic species (Saha et al., 2012; Orford et al., 2015) will benefit from decreasing crop field sizes and increasing edge density in the landscape (Hass et al., 2018). As noted by Fahrig et al. (2015), this can be accomplished without taking land out of production. In contrast, services such as the breakdown of agrichemicals and the maintenance of water quality in farmland wetlands in our region will require land use guidelines that limit the total cover of high intensity agriculture and maintain the cover of more natural areas such as forests. If, as we speculate above, the lack of edge effect on agrichemicals is due to subsurface drainage bypassing edge vegetation, it may also be possible to decrease agrichemical pollution of ditch water by restricting drain discharge through controlled tile drainage (Sunohara et al., 2015) or saturated riparian buffers (Jaynes and Isenhart, 2014).

We did not find support for the prediction that aquatic macroinvertebrate richness would be positively associated with the proportion of the landscape in forest cover. We had hypothesized, based on previous studies (Jonsen and Taylor, 2000; Didham et al., 2012), that forest cover in the surrounding landscape would facilitate movement and provide habitat for the terrestrial stages of some invertebrates. However, while some invertebrate groups respond positively to forest cover in the landscape, others can respond negatively (Frisch et al., 2016). It may be that shrubby cover (typical of field edges) is used by more semi-aquatic invertebrate taxa than forest cover as terrestrial habitat in farmland, which is consistent with our positive effect of edge cover and lack of effect of forest cover.

Our results suggest that aquatic macroinvertebrate family richness is strongly impacted by fertilizer use in our study region through direct and indirect pathways. Invertebrate richness decreased with increasing nitrogen levels in ditches (Figures 3.5 and 3.7). Richness was also indirectly negatively related to nitrogen levels (and thus also to high intensity crop cover), through a negative effect of nitrogen on dissolved oxygen levels (Figures 3.5 and 3.7). These relationships are consistent with the results from previous studies (Wang et al., 2007; Yuan, 2010). Elevated levels of inorganic nitrogen can induce toxic effects on many aquatic organisms, and also result in eutrophication, leading to harmful hypoxic or anoxic conditions (Camargo and Alonso, 2006). Considering that one third of our ditch sites had nitrate-nitrogen levels that exceeded the maximum long-term exposure level for the protection of aquatic life, it is not surprising that we found direct and indirect negative effects of nitrogen enrichment on invertebrate richness. Based on these results, we recommend that reducing nutrient losses in runoff

should be a primary goal of farmland water conservation initiatives in our region. Again, this could potentially be achieved by implementing management practices that reduce contaminant inputs from tile drain discharge (Jaynes and Isenhart, 2014; Sunohara et al., 2015).

Surprisingly, we found no relationship between macroinvertebrate family richness and pesticide levels in ditches (Figure 3.5). This is in contrast to studies that found negative effects of pesticide contamination on stream aquatic invertebrate richness (Beketov et al., 2013; Orlinskiy et al., 2015), and to research demonstrating negative effects of sublethal pesticide exposure on invertebrates (Russo et al., 2018). A possible explanation for our result is that there may be at least one species within each family that is tolerant to the pesticide levels in our ditches (e.g. Shahid et al., 2018), and so the loss of intolerant species would not result in a decrease in family richness. This explanation was also suggested by Ieromina et al. (2016), who describe an intriguingly similar result to ours: a nutrient (phosphate) was the most important predictor structuring invertebrate communities, assessed by the composition of functional traits, in agricultural ditches, while pesticides had no effect. They speculated that communities may adapt to pesticide stress through sensitive species being replaced by tolerant ones with similar functional traits, thus maintaining community trait composition.

Aquatic macroinvertebrate richness was a more sensitive water quality indicator than leaf litter decomposition or *C. dubia* population responses in bioassays. While macroinvertebrate richness responded to physicochemical water quality, leaf litter decomposition and *C. dubia* reproduction and survival did not (Figure 3.5; Appendix H). Previous studies suggested that decomposition and *C. dubia* population responses should

be sensitive indicators of water quality (Eagleson et al., 1990; Baldy et al., 2007; Medeiros et al., 2009; Piscart et al., 2009). For example, fungal decomposition of leaf litter in aquatic environments can be inhibited in low-oxygen conditions (Medeiros et al., 2009), and by nutrient enrichment (Baldy et al., 2007; Piscart et al., 2009). However, it has also been suggested that aquatic decomposer diversity can be reduced in contaminated waters without changes to decomposition rates, due to community shifts to fewer tolerant species (Pascoal et al., 2005). Additionally, moderate eutrophication in streams has been shown to accelerate decomposition, presumably by added nutrients stimulating fungal activity (Gulis et al., 2006). Regarding the lack of effects on *C. dubia*, it is possible that increases in food resources in ditch water with elevated agrichemical levels compensated for any negative toxicological effects. For example, García-García et al. (2012) suggested that elevated levels of organic matter in agricultural stream water may have augmented the diet of *C. dubia* in laboratory bioassays, leading to increases in fecundity. Thus, our results call into question the use of leaf litter decomposition and of *C. dubia* population responses in bioassays as general indicators of water quality in farmland wetlands.

Conclusions

Our results suggest that landscape management to improve farmland water quality should focus on the composition of the landscape, in particular, increasing amounts of non-crop cover such as forest, and reducing amounts of crop cover associated with high agrichemical inputs. These landscape composition predictors were strongly related to agrichemical levels in ditches, and indirectly related to aquatic macroinvertebrate richness. In contrast, landscape configurational heterogeneity (measured as the amount of

field edge cover), was positively associated with aquatic macroinvertebrate richness, but unrelated to agrichemical levels. These results imply that increasing configurational heterogeneity as a management plan for terrestrial biodiversity will also benefit aquatic macroinvertebrate diversity, but will not greatly benefit physicochemical water quality, at least in our region where tile drainage is common. The possibility that configurational heterogeneity may have stronger effects on physicochemical water quality in regions without tile drainage requires further study. Our results have practical implications for landscape management because the spatial extent in which we quantified relationships between landscape structure and water quality (1-km radius landscapes) is relevant for individual farm management. Based on our results, we recommend the creation of agri-environmental programs that encourage the preservation and enhancement of forest cover, and the reduction of crop cover associated with high chemical inputs to improve farmland water quality.

Chapter 4 – Are macroinvertebrate traits reliable indicators of specific agrichemicals?

4.1 Abstract

Determining the extent of freshwater contamination by agrichemicals is a major challenge. Biological indicators have been proposed as indirect measures of contaminants that can be used to reduce chemical monitoring costs by identifying pollution hotspots that may warrant thorough chemical testing. Many general freshwater indicators are based on taxonomic properties of aquatic macroinvertebrate communities. However, it has been suggested that metrics based on traits, rather than taxa, can be used to develop more chemical-specific and efficient macroinvertebrate indicators. Here, we investigate whether macroinvertebrate family-level traits can be used as simple indicators of elevated levels of specific agrichemical pollutants in farmland drainage ditches, to reveal areas in need of further chemical monitoring. We selected seven traits that we predicted would influence macroinvertebrate sensitivity to nitrate, to the two herbicides atrazine and glyphosate, and to neonicotinoid insecticides, and tested whether any trait-chemical relationships were strong enough to be reliable bioindicators. We collected macroinvertebrate samples and water samples for agrichemical analyses from 27 farmland drainage ditches in Eastern Ontario, Canada. We indexed the sensitivity of each sampled macroinvertebrate family to the concentration of each agrichemical, using coefficients from multiple logistic regressions of family absence/presence in the ditches on the concentrations of the four agrichemicals. We reduced the seven traits predicted to influence family sensitivity to agrichemicals to four traits, after examining their inter-dependencies. We then tested for cross-family relationships between sensitivity to each

chemical and the trait categories for each macroinvertebrate family. Two traits, oxygen acquisition and dispersal mode, were significantly associated with two agrichemical sensitivity coefficients: nitrate sensitivity was associated with mode of oxygen acquisition, with atmospheric breathers having a higher mean sensitivity coefficient than dissolved-oxygen breathers, and glyphosate sensitivity was related to dispersal mode, with passive dispersers having a higher mean sensitivity coefficient than active dispersers. However, inspection of these relationships revealed that the responses lacked enough consistency to be reliable, chemical-specific indicators. Instead, a taxa-level, post-hoc analysis indicated that further work should be conducted to determine whether there are individual taxa whose presence at a site is a strong indicator of lack or low levels of certain contaminants. In particular, the presence of Corixidae may indicate low ditch nitrate levels. Overall, however, our results combined with previous work suggest that we are unlikely to find chemical-specific indicators based on macroinvertebrates that are more efficient than a rigorous chemical sampling scheme.

4.2 Introduction

There are serious concerns about the widespread agrichemical contamination of freshwater systems (Malaj et al., 2014; Morrissey et al., 2015; Stehle and Schulz, 2015). For example, chemical contamination, primarily from pesticides, is estimated to be compromising the ecological integrity of almost half of all water bodies in Europe (Malaj et al., 2014). Commonly-used pesticides are frequency detected in surface waters at levels that impact the structure and function of aquatic ecosystems (Graymore et al., 2001; Morrissey et al., 2015; Stehle and Schulz, 2015). Eutrophication from excess nutrient loading is also a leading cause of freshwater impairment (Stendera et al., 2012).

Chemical monitoring of freshwater systems is an essential component of water quality management. However, it is expensive to thoroughly test for agrichemicals at a given site, due to the inherent temporal and spatial variation of chemicals. Agrichemical concentrations in water undergo temporal fluctuations that vary with application rates and environmental factors, such as rainfall and water chemistry (Sandín-España and Sevilla-Morán, 2012; Masters et al., 2013). Because measurements of chemical concentrations represent snapshots of conditions during the time of sampling, frequent testing is required to determine the level of contamination at a given site.

Biological indicators have been proposed as indirect measures of pollution to reduce chemical monitoring costs (Whitfield, 2001). Biological measures offer a longer temporal record of environmental conditions than chemical measures because they represent the temporally integrated conditions experienced throughout the lives of the species sampled (Whitfield, 2001; Abbasi and Abbasi, 2011). Biological indicators have

been proposed as approaches to identify "pollution hotspots" that might warrant thorough chemical testing, thus reducing the need for frequent testing at all sites (Whitfield, 2001).

Many biological indicators for freshwater are based on aquatic macroinvertebrates (Resh, 2008; Abbasi and Abbasi, 2011; Birk et al., 2012; Buss et al., 2015). Their advantages as bioindicators include their general ubiquity, abundance and diversity, and varying sensitivity to environmental perturbations (Resh, 2008; Abbasi and Abbasi, 2011). Commonly-used macroinvertebrate bioindicators are often based on taxonomic properties of the community, such as richness and diversity, relative composition of different taxa, and indices based on combined sensitivity-weighted scores for each taxon (Jones et al., 2007; Bonner et al., 2009; Abbasi and Abbasi, 2011; Laini et al., 2018).

There are potential problems with using taxonomy-based metrics as indicators of agrichemical pollution: first, these metrics often respond to environmental variables other than agrichemicals (Meyer et al., 2015; Laini et al., 2018), which reduces their reliability as indicators of these contaminants; also, it has been questioned whether they can be used to detect specific chemicals (Menezes et al., 2010; Schäfer et al., 2011; but see an evaluation of multimetric approaches in Bonada et al., 2006). Lack of chemical specificity is not an issue for the most common use of these indicators, i.e. in assessments of overall ecosystem health, rather than to detect specific chemicals. However, given the rising threats to water quality from agrichemical pollutants (Stehle and Schulz, 2015), there has been growing interest in the development of rapid bioindicators that can differentiate between chemical stressors and other human impacts, and also among chemical stressors (Culp et al., 2011; Schäfer et al., 2011; Gerner et al., 2017; Berger et al., 2018).

An additional issue with many taxonomy-based indicators is that the complexity and costs of some of the required sampling and processing protocols call into question whether they are actually more efficient than chemical sampling. Effort and cost of collecting bioindicator data can vary greatly, depending on the protocols of specific programs (Bartsch et al., 1998; Buss et al., 2015; Bo et al., 2017). Different protocols vary in the required number of samples and individuals to collect, subsampling and sorting methods, and taxonomic resolutions of identifications (Carter and Resh, 2001; Buss et al., 2015; Bo et al., 2017). Such differences can significantly affect costs and efficiency (Bartsch et al., 1998), and ultimately affect implementation and performance (Bo et al., 2017). For example, the change to a more labor-intensive and challenging biological water quality assessment method in Italy, to meet the requirements of the European Water Framework Directive, resulted in a substantial reduction in the number of sites being monitored (Bo et al., 2017).

It has been suggested that metrics based on biological traits, rather than taxa, can be used to develop more chemical-specific and efficient macroinvertebrate bioindicators (Dolédec et al., 2000; Bady et al., 2005; Schäfer et al., 2007; Culp et al., 2011; Schäfer et al., 2011; Gerner et al., 2017). Predictions can be made about which traits will be selected against by particular chemical contaminants, based on ecological theory and knowledge of chemical modes of action (Baird and Van den Brink, 2007; Baird et al., 2008; Menezes et al., 2010), with the goal of developing chemical-specific indicators (Schäfer et al., 2007; Schäfer et al., 2011; Gerner et al., 2017). Trait-based bioindicators have also been suggested to be more accurate with less sampling effort than taxonomy-based measures of invertebrate communities (Bady et al., 2005), and to require data at lower taxonomic

resolutions (e.g. family-level), than taxonomy-based measures (Dolédec et al., 2000). In addition, while taxonomy-based measures typically require abundance data (e.g. Marshall et al., 2006), trait-based measures based on presence-absence data may be effective (Gayraud et al., 2003), which could reduce sampling intensity.

Trait-chemical predictions are based on hypotheses related either to ecological sensitivity or physiological sensitivity. Ecological sensitivity is determined by a population's ability to recover from or avoid contaminant exposure (Kefford et al., 2012). For example, traits related to population growth rates influence how quickly a population recovers following pesticide exposure (Sherratt et al., 1999; Beketov et al., 2008). Populations that recover quickly are at lower risk from successive contaminant pulses than populations that recover slowly (Liess and Beketov, 2011; Kefford et al., 2012). Dispersal capabilities may also influence population recovery at impacted sites by influencing recolonization following chemical exposure (Rubach et al., 2011; Gergs et al., 2016). Dispersal may also be related to population avoidance of exposure, by allowing adult stages to escape exposure via active dispersal modes (Schäfer et al., 2011; Kefford et al., 2012). Physiological sensitivity is determined by avoidance or tolerance of chemical exposure at the organism level (Kefford et al., 2012). Two examples of traits that may influence physiological sensitivity are invertebrate body armouring and mode of respiration (Baird and Van den Brink, 2007; Rico and Van den Brink, 2015). The degree of body armouring has been negatively correlated with sensitivity to some pesticides, supporting the hypothesis that armoured organisms have lower uptake and thus lower exposure risks compared to soft-bodied organisms (Rico and Van den Brink, 2015). Respiration mode has also been associated with sensitivity, suggesting that organisms

that acquire dissolved oxygen from the water via gills or integument have higher exposure risks to chemicals than organisms that acquire oxygen directly from the atmosphere (Baird and Van den Brink, 2007; Rico and Van den Brink, 2015).

Here, we investigate whether macroinvertebrate family-level traits can be used as indicators of elevated levels of specific common agrichemical pollutants in farmland drainage ditches, a common aquatic habitat in our study region. Our overall goal is to identify simple, easy-to-use, trait-based indicators to distinguish between highly-impacted (e.g. above water quality guidelines) vs. low-impacted sites for specific agrichemicals, to reveal areas in need of further chemical monitoring. We identified traits that we predicted *a priori* would influence macroinvertebrate sensitivity to nitrate, to the two herbicides atrazine and glyphosate, and to neonicotinoid insecticides (Table 4.1). We then tested whether any trait-chemical relationships were strong enough to be informative bioindicators.

Our goals were to determine: (i) if certain traits of aquatic macroinvertebrates relate to their sensitivities to particular agrichemicals and (ii) if so, whether these relationships are strong enough and sufficiently consistent across taxa that they could be used as reliable indicators of these agrichemical pollutants in farmland water bodies.

4.3 Methods

Overview

We collected macroinvertebrate samples and water samples from 27 farmland drainage ditches in Eastern Ontario, Canada, and we measured the concentrations of four agrichemicals in the water samples. See Chapter 3.3 for full descriptions of the study

Table 4.1. Traits of aquatic invertebrates predicted to influence their sensitivity to agrichemicals.

Trait	States	Prediction	Supporting literature
Body size	*Small* = < 9 mm *Large* = > 9 mm	*Small* will be associated with higher sensitivity than *Large*	Buchwalter et al., 2002; Baird and Van den Brink, 2007; Rubach et al., 2012; Ippolito et al., 2012; Rico and Van den Brink, 2015; Mondy et al., 2016; Wiberg-Larsen et al., 2016
Degree of armouring	*Part* = body is only partly armoured or soft-bodied *All* = body completely sclerotized or hard-shelled	*Part* will be associated with higher sensitivity than *All*	Rico and Van den Brink, 2015
Feeding guild	*Filter-feeder* = filters small particles from water *Other* = primarily an herbivore or scavenger via scraper, grazer, or collector-gatherer feeding guilds *Predator* = primary feeding guild is predator	*Filter-feeder* will be associated with highest sensitivity and *Predator* the least	Hartman and Martin, 1984; Baird and Van den Brink, 2007; Ippolito et al., 2012; Rubach et al., 2012; Liess et al., 2017
Habit	*Burrower* = burrows in substrate *Crawler* = climbs, crawls, or attaches to substrate/submerged materials *Swimmer* = primary habit is swimming in water column or skating on surface	*Burrower* and *Crawler* will be associated with higher sensitivity than *Swimmer*	Lange et al., 2014; Berger et al., 2018

Oxygen acquisition	*Water* = dissolved oxygen acquired from water via gills or cutaneous respiration *Atmosphere* = at least one aquatic life cycle stage acquires oxygen from atmosphere	*Water* will be associated with higher sensitivity than *Atmosphere*	Buchwalter et al., 2002; Baird and Van den Brink, 2007; Rico and Van den Brink, 2015; Mondy et al., 2016
Dispersal mode	*Passive* = passively disperses via wind, drift, or animal vectors *Active* = actively disperses via flight, swimming, or crawling	*Passive* will be associated with higher sensitivity than *Active*	Kefford et al., 2012; Gergs et al., 2016; Mondy et al., 2016
Voltinism	*Univoltine* = Taxa has ≤ 1 generation per year *Multivoltine* = Taxa has > 1 generation per year	*Univoltine* will be associated with higher sensitivity than *Multivoltine*	Sherratt et al., 1999; Beketov et al., 2008; Liess and Beketov, 2011; Kefford et al., 2012; Lange et al., 2014; Gergs et al., 2016; Mondy et al., 2016

sites, macroinvertebrate and water sampling, and agrichemical analyses. We indexed the sensitivity of each sampled macroinvertebrate family to the concentration of each agrichemical, using coefficients from multiple logistic regressions of family absence/presence in the ditches on the concentrations of the four agrichemicals. We then selected seven traits predicted to influence aquatic macroinvertebrate family sensitivity to agrichemicals, which we subsequently reduced to four traits after examining their inter-dependencies. We tested for cross-family relationships between sensitivity to each chemical (the coefficients from the multiple logistic regressions) and the trait categories for each macroinvertebrate family. For each significant trait-sensitivity relationship, we evaluated its strength and degree of consistency across families, to determine whether the relationship could be used as a reliable, chemical-specific indicator.

Are macroinvertebrate traits related to family sensitivities to particular agrichemicals?

We selected seven traits (Table 4.1) that we predicted would be important determinants of aquatic macroinvertebrate sensitivity to the agrichemicals measured in ditch water, and assigned each family a particular state for each trait (Appendix I). We acquired most of the family trait information from a trait database developed by Vieira et al. (2006) for North American lotic invertebrates. We supplemented any missing trait data from this database with information from other sources in the literature (See Appendix I for family trait state assignments and sources), including trait databases constructed by Poff et al. (2006) and Schriever and Lytle (2016). We used a "majority rules" approach (as in Poff et al., 2006) to assign states to families represented multiple times in the database by different taxonomic groups within a family. We also used this approach for occasions when individual taxa (e.g. a species) had multiple records with

conflicting state entries, and also ensured that individual taxa were only represented once in family trait assignments. If the different states for a given trait had proportionally equal representation by different taxa within a family, we assigned the family the state that we predicted would be associated with higher agrichemical sensitivity.

To avoid having redundant traits, we assessed pairwise dependencies between traits across families using chi-square or Fisher's exact tests (when expected frequencies were < 5) and reduced the number of traits by excluding ones that were strongly associated with others, while keeping the ones for which we had the strongest biological hypotheses and/or had the strongest evidence from existing literature of a likely association between that trait and sensitivity to agrichemicals.

To calculate relative measures of invertebrate family sensitivities to each agrichemical, we modeled the presence/absence of each family at a site using the four agrichemicals as predictor variables in a generalized linear model with the binomial distribution and logit-link function, and used the resulting coefficients as indices of sensitivity. We coded absence and presence as 1 and 0, respectively, so that a large positive coefficient could be interpreted as a high probability of absence at sites with high chemical concentration, i.e. a strong sensitivity to the chemical. We limited this modeling to families with < 90% and > 10% incidence, resulting in 35 families with sufficient incidence data. The four agrichemical predictors were log-transformed to normalize distributions and homogenize variances prior to statistical analyses, and standardized to a mean of 0 and SD of 1 to allow for direct comparison of coefficients. Agrichemical levels reported as less than the limit of detection (LOD) were set to LOD/2. Pairwise correlations between agrichemical predictors were all < 0.6 (Appendix J), which is below

the accepted maximum collinearity thresholds for estimating independent effects of predictors ($r < 0.7$; Dormann et al., 2013).

To test our predictions about the associations between invertebrate traits and sensitivity to individual agrichemicals, we used the four agrichemical sensitivity coefficients across families from the binomial models as continuous response variables, and modeled the relationships between these and the selected categorical traits using linear models. We checked that model assumptions were met by examining residual plots and with Levene's test to confirm homogeneity of variances for each trait variable in each model, using the package car (Fox and Weisberg, 2011). We found no evidence of any model assumption violations. We conducted all analyses in R 3.5.1 (R Core Team, 2018).

Are trait-sensitivity relationships strong enough and sufficiently consistent across taxa that they could be used as chemical-specific indicators of agrichemical pollutants?

To determine if any trait-sensitivity relationships were strong enough to suggest any particular traits as reliable bioindicators of particular agrichemicals, we compared the ranges of sensitivity coefficients between the trait states of any significant trait-agrichemical sensitivity relationships, to see if they were consistent enough across taxa to allow a person assessing ditch water quality to make a reliable evaluation, based on observing that trait at a site. We reasoned that, to be a reliable indicator, there should be a high degree of consistency among the sensitivity coefficients for a given state, i.e. all families classified under the state should have coefficients with the same direction of effect and most coefficients should be > 1 standard deviation from the mean.

Post hoc: Are some families sensitive enough to use as indicators?

Given our small number of sites (n = 27), we did not expect to find strong relationships between individual taxa and chemical predictors; however, because the ranges of sensitivity coefficients within particular traits suggested that some trait-sensitivity relationships were driven by a few taxa, we were prompted to evaluate, post-hoc, the potential value of individual families as indicators of particular agrichemicals. We calculated 95% confidence intervals around each standardized agrichemical sensitivity coefficient for each family from the binomial models, and considered a family to be a potentially useful indicator of a particular contaminant if there was strong statistical support for the relationship, evidenced by a coefficient with 95% confidence intervals that excluded zero. We limited this assessment to families that had > 30% and < 70% presences or absences across the study sites, to avoid including potentially spurious relationships that can occur in logistic regressions with small sample sizes and uneven representation of states.

4.4 Results

Are macroinvertebrate traits related to family sensitivities to particular agrichemicals?

Elimination of correlated traits

We reduced the number of traits from seven to four, based on pairwise dependencies between traits (Table 4.2; Appendix K). Strong associations between four pairs of traits (feeding guild and dispersal mode, feeding guild and body size, voltinism and body size, and habit and oxygen acquisition) led us to eliminate body size, feeding guild, and habit, and retain degree of body armouring, oxygen acquisition, dispersal

Table 4.2. Strength of pairwise dependencies between traits, measured with Cramer's V. Asterisks denote statistical significance ($p < 0.05$ or $p < 0.001$) determined by chi square tests or Fisher's exact tests for comparisons with > 20% expected frequencies < 5.

Trait	Size	Armour	Respiration	Feed	Habit	Voltinism	Dispersal
Size	-	0.01	0.30	0.51**	0.34	0.42*	0.07
Armour		-	0.06	0.09	0.01	0.31	0.17
Respiration			-	0.13	0.42	0.11	0.06
Feed				-	0.16	0.19	0.44*
Habit					-	0.06	0.16
Voltinism						-	0.08
Dispersal							-

mode, and voltinism for agrichemical sensitivity analyses. We chose to keep voltinism over body size, and dispersal mode over feeding guild, because voltinism and dispersal mode are less confounded with other traits than are body size and feeding guild (Table 4.2; also see Poff et al., 2006). We selected oxygen acquisition over habit because there is more support in the literature for oxygen acquisition as a trait that influences invertebrate pollution sensitivity (e.g. Baird and Van den Brink, 2007) than habit.

Macroinvertebrate family sensitivities to agrichemicals

Standardized nitrate sensitivity coefficients from the binomial models across the 35 taxa ranged from -2.11 to 1.60 (mean -0.09 ± 0.70 SD), atrazine coefficients ranged from -1.51 to 2.87 (mean 0.16 ± 0.72 SD), glyphosate coefficients ranged from -1.56 to 1.59 (mean -0.11 ± 0.76 SD), and neonicotinoid sensitivity coefficients across the 34 taxa ranged from -3.08 to 3.05 (mean -0.07 ± 1.12 SD) (Appendix L). Pairwise correlations across families between the four sensitivity coefficients are shown in Figure 4.1. Neonicotinoid sensitivity coefficients had significant negative correlations with both atrazine and glyphosate sensitivity coefficients, indicating that taxa sensitive to neonicotinoid insecticides tend to be insensitive to the two herbicides, and vice versa.

Relationships between sensitivity coefficients and macroinvertebrate family traits

Two traits, oxygen acquisition and dispersal mode, were related to two agrichemical sensitivity coefficients (Figure 4.2). Sensitivity to nitrate was significantly associated with mode of oxygen acquisition, with atmosphere breathers having a higher mean sensitivity coefficient than water breathers ($t = -2.31$, $p = 0.03$, $df = 30$). Glyphosate sensitivity was significantly related to dispersal mode, with passive dispersers

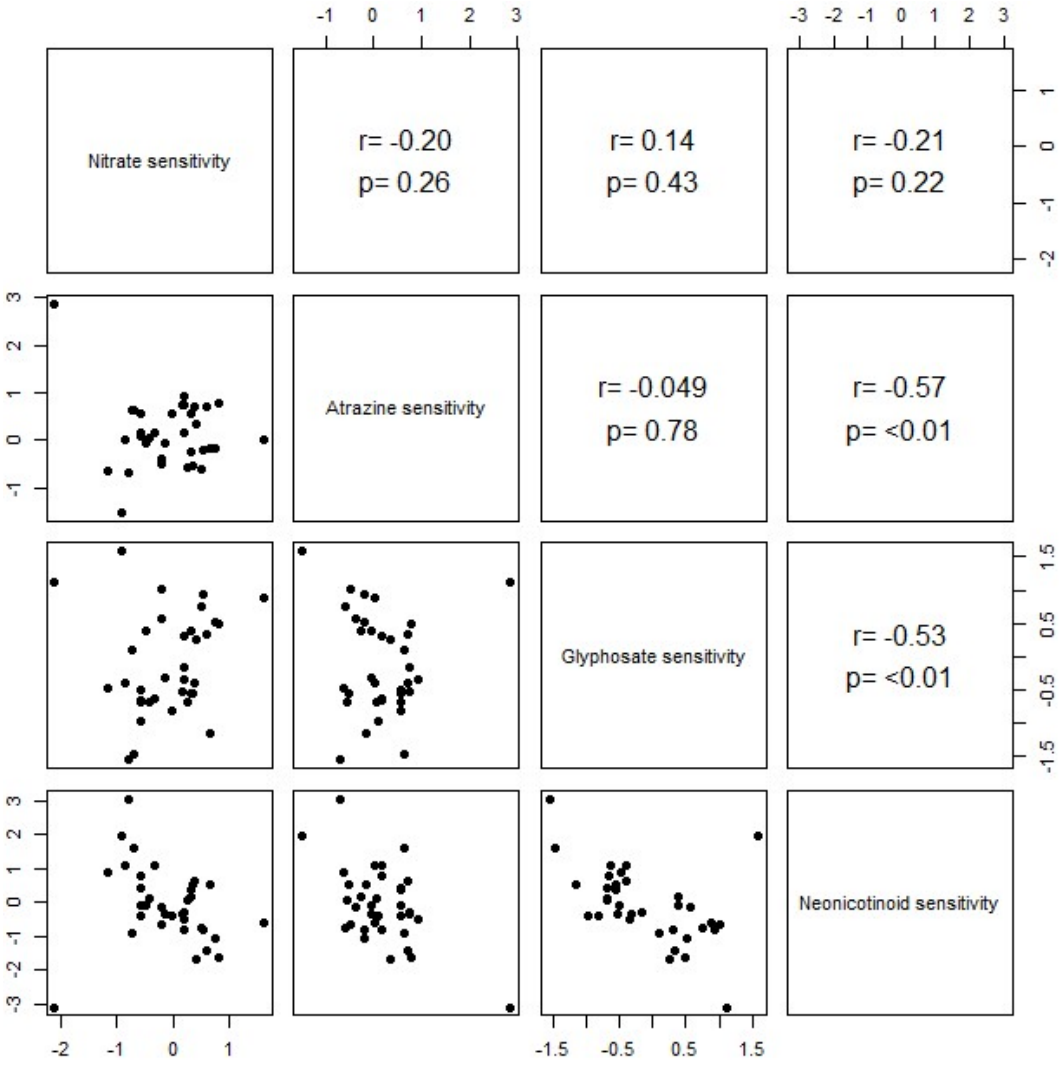

Figure 4.1. Cross-family pairwise correlations of sensitivities to four agrichemicals. Sensitivities are standardized coefficients from binomial generalized linear models of family absence/presence on concentrations of nitrate, atrazine, glyphosate, and neonicotinoid insecticides.

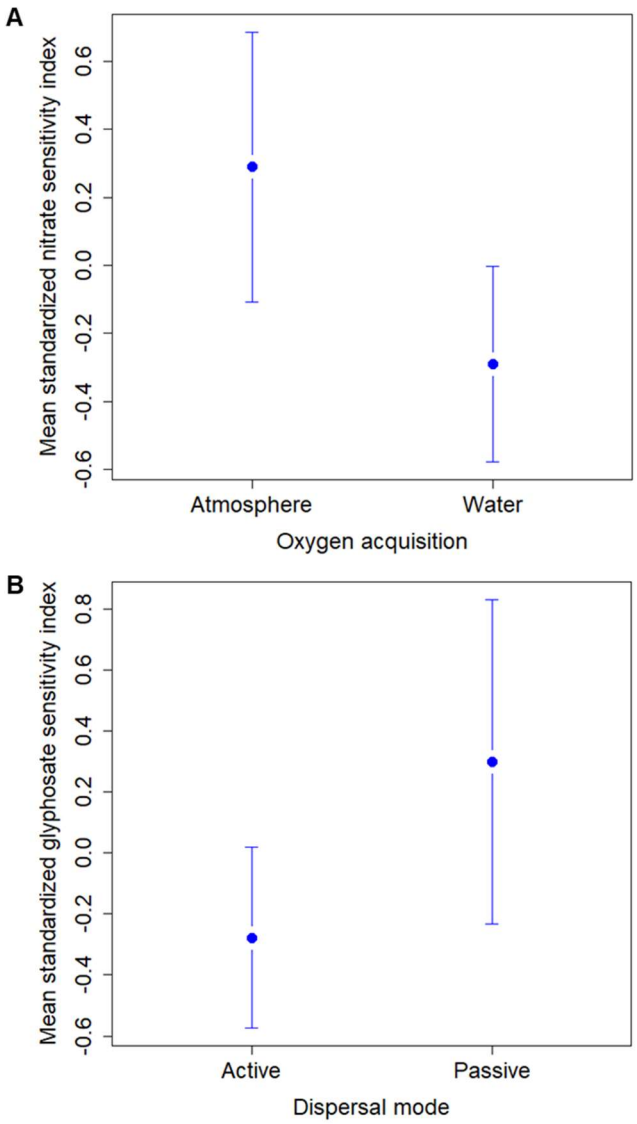

Figure 4.2. Significant relationships between macroinvertebrate family traits (n = 35 families) and agrichemical sensitivities. Sensitivities are standardized coefficients from binomial generalized linear models of family absence/presence on concentrations of the four agrichemicals. (a) Families that acquire oxygen directly from the atmosphere have a higher mean sensitivity coefficient to nitrate concentration, i.e. a higher probability of absence with increasing nitrate levels, than families that acquire oxygen from the water.

(b) Families that disperse passively have a higher mean sensitivity coefficient to glyphosate concentration, i.e. a higher probability of absence with increasing glyphosate levels, than families that disperse actively.

having a higher mean sensitivity coefficient than active dispersers ($t = 2.10$, $p = 0.04$, $df = 30$).

Are trait-sensitivity relationships strong enough and sufficiently consistent across taxa that they could be used as chemical-specific indicators of agrichemical pollutants?

Inspection of significant trait-sensitivity relationships revealed that the responses of atmosphere vs. water breathers to nitrate, and passive vs. active dispersers to glyphosate lacked enough consistency to be reliable indicators (Figures 4.3 and 4.4). Although the mean standardized nitrate coefficient for atmospheric-breathing families was higher than the mean standardized coefficient for families that acquire oxygen from the water (Figure 4.2a), and the mean standardized glyphosate coefficient for passive dispersers was higher than the mean standardized coefficient for families that actively disperse (Figure 4.2b), the direction of the coefficients was highly inconsistent across families in both cases. Three atmospheric breathing families (Culicidae, Nepidae, and Planorbidae) had negative nitrate sensitivity coefficients, i.e. absence was more strongly associated with decreasing levels (Figure 4.3), and three passive dispersers (Sphaeriidae, Valvatidae, and Hyalellidae) had negative glyphosate sensitivity coefficients (Figure 4.4). Furthermore, most of the positive coefficients for each group were < 1 standard deviation from the mean, suggesting that the relationships between oxygen acquisition and nitrate sensitivity, and between dispersal mode and glyphosate sensitivity, were driven by a few families in each group.

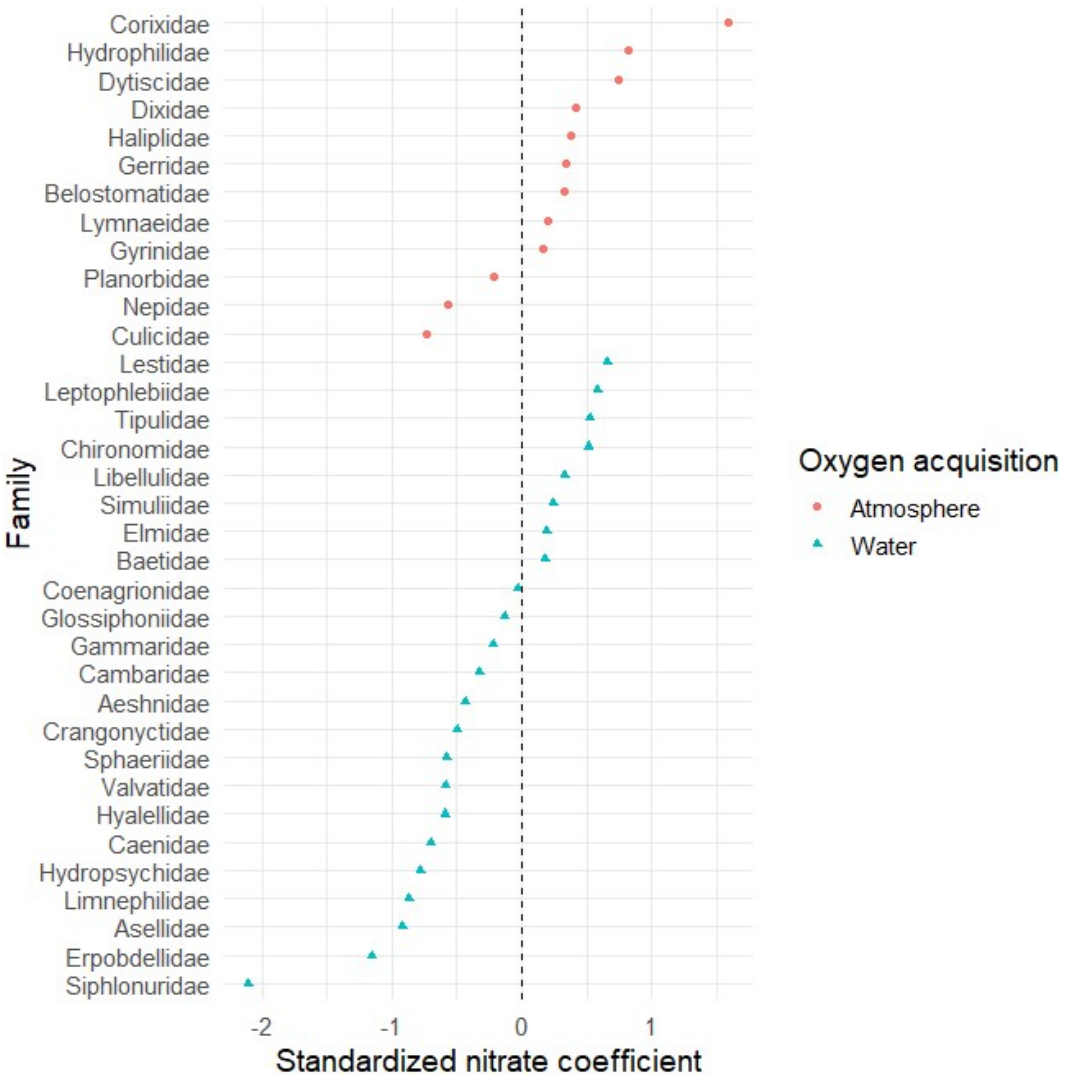

Figure 4.3. Standardized nitrate coefficients from each macroinvertebrate family binomial generalized linear model, showing families that acquire oxygen from the atmosphere vs. from the water. While the mean nitrate coefficient for atmospheric-breathing families is higher than the mean coefficient for families that acquire oxygen from the water (Figure 4.2a), only 75% of atmospheric breathers have positive coefficients, and most are < 1 standard deviation from the mean, suggesting that the relationship between oxygen acquisition and nitrate sensitivity is driven by only a few families in each group.

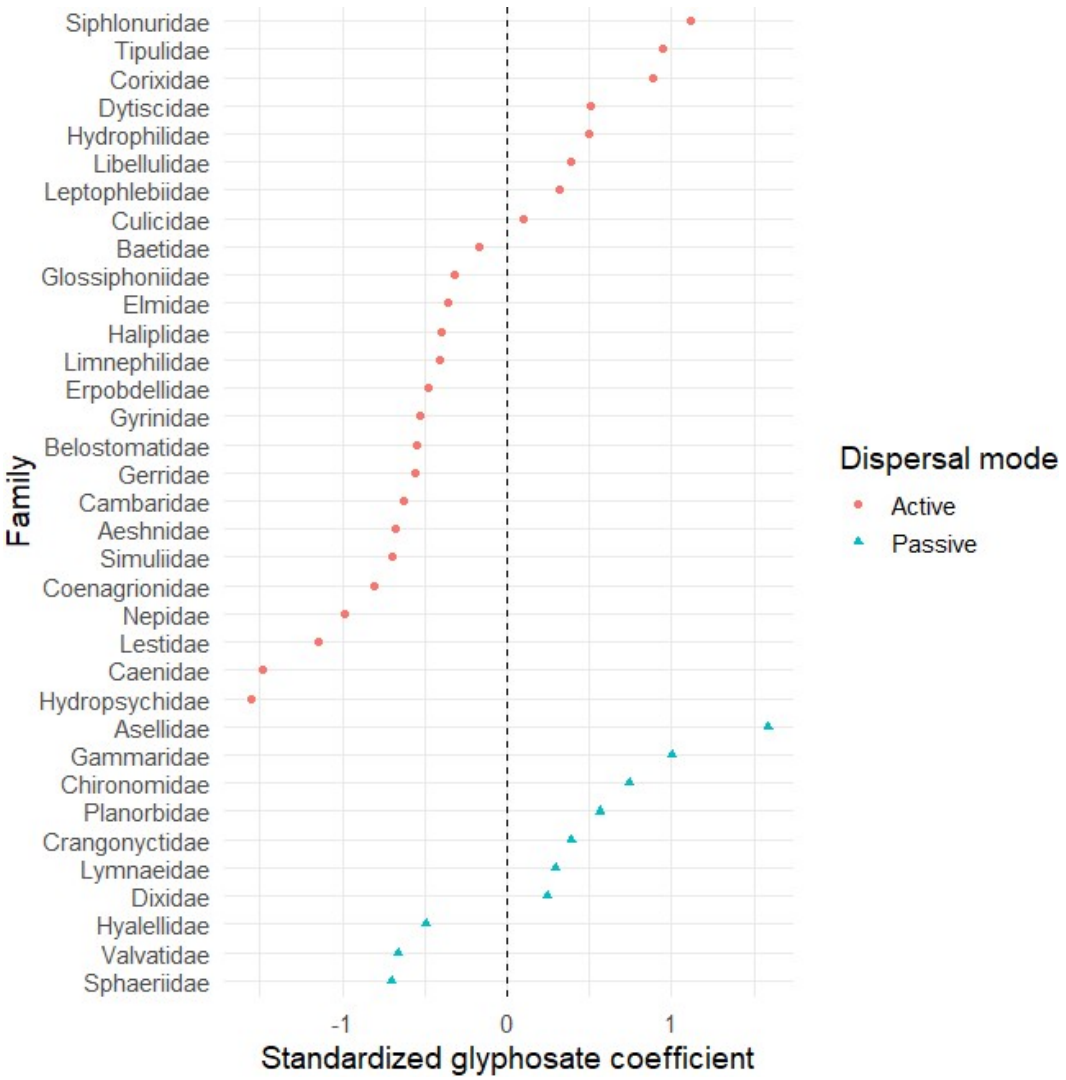

Figure 4.4. Standardized glyphosate coefficients from each macroinvertebrate family binomial generalized linear model, showing families that have active vs. passive dispersal modes. While the mean glyphosate coefficient for passive dispersers is higher than the mean coefficient for families that actively disperse (Figure 4.2b), only 70% of passive dispersers have positive coefficients, and most coefficients are < 1 standard deviation from the mean, suggesting that the relationship between dispersal mode and glyphosate sensitivity is driven by only a few families in each group.

Are some families sensitive enough to use as indicators?

Three families exhibited strong responses, i.e. had sensitivity coefficients with 95% confidence intervals that excluded zero, to two agrichemicals (Appendix L). The probability of Corixidae absence at a site increased with increasing nitrate concentrations, and both Asellidae and Hydropsychidae absences were positively associated with total neonicotinoid levels (Figure 4.5). However, Asellidae was relatively common across the study sites, with only four absences, and Hydropsychidae was rare, with only three presences (Figure 4.5). This uneven representation of absences and presences means that we are not confident in the apparent strength of these relationships, at least not confident enough to indicate that these taxa could be used as reliable indicators. On the other hand, Corixidae had relatively equal representation of presences and absences (66.7% and 33.3%, respectively), giving us some confidence in its usefulness as an indicator taxon of nitrate enrichment. The Corixidae binomial model predicts that there is only a 1% probability of Corixidae being absent at a site at the lowest recorded nitrate level (LOD/2 = 0.05 mg/L), and an 82% probability of absence at the highest measured nitrate level (9 mg/L), while holding the other three chemical predictors at their mean values.

4.5 Discussion

Our results indicate two relationships between macroinvertebrate traits and agrichemical contamination of ditches. First, passive dispersers are on average more sensitive to glyphosate than active dispersers; second, atmospheric breathers are absent from nitrate-enriched ditches more often than taxa that breath dissolved oxygen (Figure 4.2). The relationship between dispersal mode and glyphosate sensitivity supports the prediction that passive dispersers are more sensitive, at least to glyphosate pollution, than

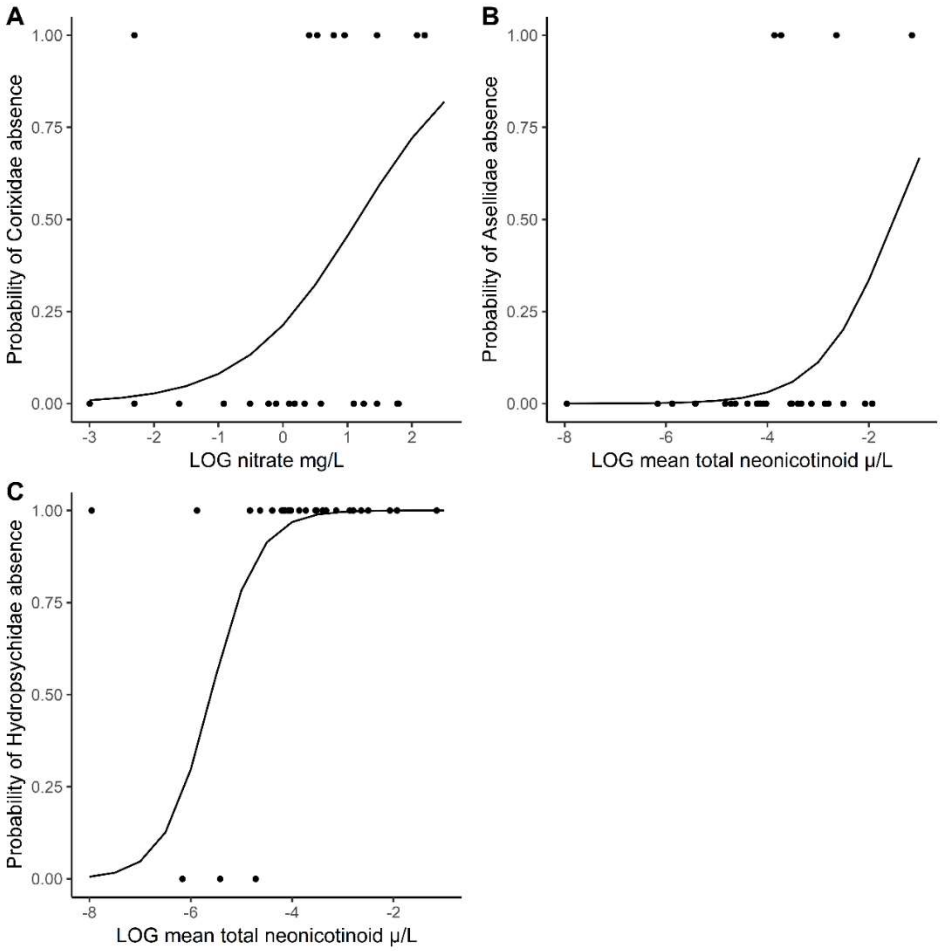

Figure 4.5. Relationships between potential indicator macroinvertebrate families and two agrichemicals. (a) Predicted probability of Corixidae absence at a site with nitrate concentration, while holding atrazine, glyphosate, and total neonicotinoid concentrations at their mean measured values. (b) Predicted probability of Asellidae absence at a site with total neonicotinoid concentration, while holding nitrate, atrazine, and glyphosate at their mean measured values. (c) Predicted probability of Hydropsychidae absence at a site with total neonicotinoid concentration, while holding nitrate, atrazine, and glyphosate at their mean measured values.

active dispersers, suggesting that taxa with active dispersal modes have either higher population recovery rates or population avoidance potential than passive dispersers. The relationship between atmospheric breathers and nitrate sensitivity was surprising, given that atmospheric breathers are typically recognized as being more tolerant to pollution, particularly nutrients (e.g. Verdonschot et al., 2012; Mondy et al., 2016), than dissolved oxygen-breathing organisms (Resh et al., 2008). It is possible that most of the dissolved oxygen-breathing invertebrates inhabiting farmland ditches have physiological or behavioural adaptations that allow them to tolerate high nitrogen and associated low-oxygen conditions. For example, a high internal oxygen regulation capacity allows an Asellid species to tolerate hypoxic conditions (Rotvit and Jacobsen, 2013), and many dissolved oxygen-breathing aquatic insects can perform specialized gill or cutaneous ventilation behaviours to help meet their oxygen demands (Resh et al., 2008). It is also possible that certain types of atmospheric breathing are more sensitive than others to low-oxygen conditions. For example, Berger et al. (2018) found similar unexpected relationships between plastron respiration (a type of air-bubble breathing) and sensitivity to water chemistry variables, including nitrate. While respiratory bubbles are initially acquired from the atmosphere to serve as underwater oxygen supplies, they also function as gills by exchanging oxygen and carbon dioxide with the surrounding water (Resh et al., 2008; Matthews and Seymour, 2010; Goforth and Smith, 2012). Low-oxygen conditions can impair such functioning (Hinton, 1976; Resh et al., 2008; Goforth and Smith, 2012), thus necessitating more frequent risky and energetically-costly trips to the surface to renew oxygen stores. Elevated nitrate levels may therefore negatively affect respiratory bubble-breathers by resulting in low-oxygen conditions that disrupt bubble

functioning. A post-hoc analysis of our data supports this hypothesis: bubble breathers had significantly higher nitrate sensitivity coefficients than other atmospheric breathers (e.g. siphon breathers) or dissolved-oxygen breathers (Appendix M).

Although we detected relationships between traits and sensitivity to chemical contamination, these relationships are not consistent enough to develop reliable bioindicators (Figures 4.3, 4.4). Trait-based bioindicator approaches based on multiple traits have found similar mixed results in regards to chemical specificity (Schäfer et al., 2011; Rico et al., 2016; Berger et al., 2018; Weber et al., 2018; Lemm et al., 2019). While we do not dispute that macroinvertebrate community metrics are valuable ecosystem-quality indicators used in many biomonitoring programs (e.g. Jones et al., 2007; Birk et al., 2012), we were looking for simple relationships where the complete presence or absence of a trait category could clearly indicate elevated levels, or very low levels, of a particular agrichemical contaminant. This lack of consistency could be caused by associations between traits and other environmental factors, such as other aspects of water chemistry and the physical habitat (Vieira et al., 2006; Berger et al., 2018). For example, the absence of a trait category could occur in clean water due to a habitat feature such as channel morphology. Another cause for the lack of consistency could be due to inaccuracies in family trait assignments, particularly when taxa within a family exhibit different trait characteristics. While we used a “majority rules” approach to assign family trait states, it is possible that some families in our study system are mostly represented by taxa with the less-common trait states. Accurately assigning trait states can also be complicated for taxa that exhibit flexibility in trait expression under different

circumstances, e.g. some taxa may be considered either omnivorous or predaceous, depending on available resources.

The lack of consistency between traits and chemical sensitivity could also be due to trait intercorrelations, which can potentially complicate interpretations of bioindicator responses (Vieira et al., 2006; Berger et al., 2018). For example, an association between a chemical and particular trait may cause correlated traits to also respond, or associations could be masked or diluted if correlated traits respond in opposite ways. We found strong relationships between 4 pairs of our original 7 traits (Table 4.2), indicating that the particular trait-sensitivity relationships we found could have been driven by other traits. For example, the relationship between dispersal mode and glyphosate sensitivity could have been influenced by feeding guild, as all active dispersers in our dataset were predators (Appendix K). Berger et al. (2018) also suggest that trait responses to chemicals can be confounded by taxonomy. For example, they speculate that an observed relationship between larger body size and chemical tolerance could have been driven by the taxonomic class Gastropoda, as they identified most Gastropods in their dataset as both large and generally tolerant of contamination.

While we are not able to identify trait bioindicators for specific chemicals, our taxa-level post-hoc analysis indicates that further work should be conducted to determine whether there are individual taxa whose presence at a site is a strong indicator of lack or low levels of particular chemicals. Our post-hoc analysis suggests that the presence of Corixidae may indicate low ditch nitrate levels (Figure 4.5; Appendix L). This was surprising, because some important indices of general water quality exclude Corixidae, and Hemipterans in general, because the organisms are not dissolved-oxygen breathers

and are therefore assumed not to be influenced by dissolved oxygen levels and associated eutrophic conditions (e.g. Hilsenhoff, 1982). It is possible that Corixidae is more sensitive to nitrate and associated low-oxygen conditions than previously thought, due to negative effects on their respiratory bubbles, as discussed above. Another possible explanation is that Corixidae is responding to nitrate-induced effects on aquatic plant communities, such as reductions in submerged macrophytes, as described in Dalton et al. (2015). Interestingly, this would suggest that potential indicator taxa can be organisms that actively avoid certain habitat changes in response to a chemical and not to the chemical itself. It is also of interest to note that our Corixidae-nitrate data indicate that the Canadian water quality guidelines for maximum nitrogen levels for the protection of aquatic life should likely be reduced. Using the indicator value index (De Cáceres and Legendre, 2009) and a lower benchmark for total nitrogen (1.68 mg/L; Morgan and Kline, 2011), Corixidae emerged as a significant indicator of nitrogen, but not while using the Canadian water quality guidelines long-term exposure maximum level for nitrate-nitrogen (3 mg/L). Our post-hoc analysis also suggested that Asellidae and Hydropsychidae could be indicators of elevated glyphosate levels; however, we have less statistical confidence in these results.

It is also possible that other organism groups might be useful indicators of specific chemicals in farm wetlands. For example, plant and algae indicators are commonly used in assessments of general ecosystem condition (Barbour et al., 1999; Birk et al., 2012). They have also been proposed as potentially sensitive indicators of agriculture-related pollutants, such as nutrients and herbicides (Barbour et al., 1999; Melzer, 1999; Potapova and Charles, 2007; Resh, 2008). Plants, in particular, have been

suggested to be good indicators of eutrophic conditions (Melzer, 1999; Penning et al., 2008). In our study area, aquatic plant communities were previously shown to be negatively affected by nitrate levels while being uninfluenced by atrazine concentrations (Dalton et al., 2015). Increasing nitrate concentrations were associated with reductions in submerged macrophytes and increases in non-native species (Dalton et al., 2015). Another promising organism group are bacteria: certain taxa have been identified as potentially useful indicators of pollution gradients in an area of high anthropogenic disturbance (Li et al., 2019). Given their usefulness as general disturbance and pollution indicators, the potential for true chemical stressor-specificity in these alternative groups warrants further investigation.

Overall, our results combined with previous work suggest that we are unlikely to find chemical-specific indicators based on macroinvertebrates that are more efficient than a rigorous chemical sampling scheme. Reliable, chemical-specific macroinvertebrate bioindicators will likely need to be complex or may not be possible due to the influence of multiple, often correlated, factors as discussed above. Instead, focusing efforts on more cost-effective chemical sampling would be beneficial for management of specific chemicals in highly-modified farm wetlands. The development of passive sampling techniques are particularly promising as accurate, simple, and low-cost chemical monitoring options (Zabiegała et al., 2010; Valenzuela et al., 2019).

Chapter 5 – General Discussion

My goal for this thesis was to uncover relationships between landscape structure and aquatic ecosystems that could be used to provide management options in farmland conservation efforts. In this general discussion, I am going to review my major findings and explore how they could be incorporated in existing agri-environmental policies and programs, and make recommendations for future management directions.

Taken together, the results of my thesis suggest that landscape management to support farmland aquatic ecosystems should focus mainly on landscape composition, rather than configuration. In particular, such management should aim to increase or maintain amounts of non-crop cover such as forest, and reduce or maintain low amounts of crop cover with high agrichemical inputs. There are many more agri-environmental policies and programs that support increasing or maintaining areas of natural or semi-natural cover in agricultural landscapes, than ones that focus on reducing or maintaining low amounts high-intensity crop cover. For example, the Alternative Land Use Services (ALUS), a Canadian non-profit organization, has supported projects to establish areas of native vegetation, such as tallgrass prairie plants, on individual farms (ALUS, 2017). There are also land retirement programs such as the Conservation Reserve Program in the United States and "out-of-production" schemes in the European Union that pay farmers to remove working land from production and restore natural cover for contractual periods of time (Baylis et al., 2008; Keenleyside et al., 2011; USDA FSA, 2019). Most of these programs, however, focus on herbaceous cover types, rather than forest. The only examples of policies and programs that relate to reducing amounts of high-intensity crop cover are ones that focus on maintaining areas of low-intensity agriculture, such as

traditional pasture lands and organic farming systems (Baylis et al., 2008; Keenleyside et al., 2011; Batary et al., 2015). Such whole-farm management approaches, which are distinct from ones that target individual management actions, are much more common in the European Union, where cultural and aesthetic attributes of traditional farmland are highly valued (Baylis et al., 2008; Keenleyside et al., 2011; Batary et al., 2015). Promoting the management recommendations based on my results therefore requires a need for more agri-environmental policies in Canada that are focused on protecting and enhancing forested cover, and low-intensity agricultural systems.

My results also suggest that increasing landscape configurational heterogeneity benefits aquatic biodiversity in agricultural landscapes. In particular, my results indicated that anuran total abundance and aquatic macroinvertebrate richness can be enhanced by reducing crop field sizes/increasing edge density in farmland. These conclusions support the creation and promotion of agri-environmental policies and programs that encourage the preservation of vegetated margins, such as hedgerows, and maintenance of small field sizes in agricultural landscapes – which is contrary to the current trends towards hedgerow removal and field enlargement (Robinson and Sutherland, 2002; Rodríguez and Wiegand, 2009; Jeswiet and Hermsen, 2015). Increasing configurational heterogeneity as a management strategy matches many of the out-of-production schemes in the European Union that focus directly on maintaining and managing hedgerows (Keenleyside et al., 2011), as well as ones that focus on preservation of traditional agricultural landscapes, which are typically more spatially heterogeneous than modern, conventional farmland (Robinson and Sutherland, 2002; Batary et al., 2015). There are a few limited examples of such initiatives in North America. For example, in Canada,

ALUS and the provincial government of Prince Edward Island have supported hedgerow planting on individual farms for the provision of multiple ecosystem services (ALUS, 2017; Government of Prince Edward Island, 2019), and there have also been some farmland conservation programs that supported the planting of in-field vegetated corridors as management actions for particular species (e.g. the BadgerWay Program in Ontario; SARPAL, 2018). However, such programs have been very limited and local. Given that my results combined with previous work suggest that increasing farmland configurational heterogeneity enhances biodiversity of a range of taxa (e.g. Fahrig et al., 2015), there is a need for policies and programs that are designed to increase configurational heterogeneity, by encouraging small field sizes or maintaining field edge cover as clear management options for biodiversity enhancement.

The results of my thesis also suggest that reducing nutrient losses in farm runoff should be a primary goal of water quality management in our region. This is one of the most commonly addressed environmental goals in agri-environmental programs (e.g. CAP, 2019). Current legal requirements in Ontario related to agri-environmental management include the requirement for large livestock farms to establish approved nutrient management plans, and for the establishment of vegetated buffer zones around surface waters within or adjacent to fields that receive nutrient applications (Government of Ontario, 2002). Indeed, the establishment of vegetated buffers is one of the most common management options supported by agri-environmental policies and programs in various countries to reduce soil and nutrient losses by surface runoff (Keenleyside et al., 2011; Corry, 2014; CAP, 2019). Land retirement is also supported by some programs as a means to reduce soil erosion and associated nutrient losses (Cory, 2014; USDA FSA,

2019; CAP, 2019). In 2017, ~94 700 km^2 of land enrolled in the Conservation Reserve Program was estimated to reduce losses by > 174 billion kg of sediment and > 236 million kg of nitrogen from farmland (CRP, 2017). Other common soil and nutrient management options supported by programs include promoting conservation tillage (i.e. no-tillage) and use of precision agriculture technologies (Keenleyside et al., 2011; Corry, 2014). The Environmental Farm Plan in Canada, for example, through partnerships with multiple agencies, has supported cost-sharing opportunities for such strategies (EFP, 2016). Despite these efforts, however, nutrient pollution from farm runoff is a still a significant water quality problem in Ontario (e.g. Eimers and Watmough, 2016), suggesting a need for stronger management actions. Furthermore, while practicing no-tillage was previously assumed to reduce nitrogen losses via reduced soil erosion, recent work has confirmed that it generally results in higher nitrate losses from fields (Daryanto et al., 2017). Alternative options to incorporate in more nutrient management policies and programs could include support for the establishment of saturated riparian buffers (Jaynes and Isenhart, 2014), installations of controlled tile drainage valves on drain outlets (Sunohara et al., 2015), and intercropping with perennial cover (Corry, 2014), as well as further investment in precision agriculture technologies to better manage nutrient applications.

Taken together, the results of my thesis also suggest that, while invertebrates were sensitive to farmland water quality, they are probably less efficient than rigorous chemical sampling for the monitoring of specific contaminants. Such sampling is needed, considering the elevated levels of nitrate, atrazine, and total neonicotinoids detected in some of my ditch water samples. Indeed, while a recent assessment concluded that

pollution, particularly from agriculture, is a serious threat to Canadian surface waters, it also identified data deficiency as a major problem, as most of the sub-watersheds in the country lacked enough biological and/or chemical water quality data to be able to make baseline ecosystem assessments (WWF, 2017). My results support the need for both aquatic invertebrate and chemical data to make complete water quality assessments. Currently, such data exists across the country as a hodgepodge of inconsistent, often incompatible, datasets collected by various governmental, non-profit, and private agencies, that are often difficult to find or inaccessible (WWF, 2017). While other countries, such as the United States, provide national oversight to ensure that routine surface water quality assessments comply with requirements to collect biological and chemical data (Govenor et al., 2017), Canada lacks such federal government guidance and enforcement of standards (Schlindler, 2019). Thus, there is a critical need for national water policies that mandate data standardization and centralization, enforcement of water quality standards, and increased routine sampling and chemical testing.

The development and application of more effective policies to support aquatic biodiversity and protect water quality in farmland will require significantly more funding and program participation (Howe, 2017). Ultimately, the most fundamental challenge in achieving environmental goals through policies is the lack of adequate funding. The vast majority of government support for agriculture is directed into business risk management programs, which provide income subsidies and crop insurance payments to farmers (Corry, 2014; Eagle et al., 2015; Blandford and Matthews, 2019). For example, funding for environmental programs only accounted for 1.32% of total money spent on agricultural programs in Canada from 2003–2010 (Eagle et al., 2015). In addition to

monopolizing the lion's share of government funding, agricultural business support policies may, under some circumstances, be associated with higher rates of environmental degradation (Eagle et al., 2015). Linking income support to environmental stewardship through policy can be an effective method to increase adoption of better management practices and achieve positive environmental outcomes (Aviron et al., 2008; Herzog et al., 2008). While generally absent from Canadian policies, such cross-compliance measures are common throughout Europe and have been shown to be successful in promoting farmland biodiversity (Aviron et al., 2008) and reducing nutrient pollution of surface water (Herzog et al., 2008). Another strategy proposed to encourage adoption of best management practices is to promote non-crop rural industries that can supplement farm incomes, such as renewable energy generation (Corry, 2014). As it has been suggested that adoption of best management practices can be positively related to income stability (Filson, 2011; Corry, 2014), finding ways to stabilize farm incomes without intensifying agricultural production could be a way to encourage environmental stewardship (Corry, 2014).

In conclusion, I have identified agri-environmental policy options that should be promoted, based on the results of my thesis, to support farmland aquatic ecosystems. In particular, there is a need for more policies and programs that encourage the preservation and establishment of areas of forested cover, such as woodlots, and areas of low-intensity agriculture, such as forage crops and organic production, and features associated with configurational heterogeneity, such as hedgerows, in farmland. There is also a need for stronger policies and more diverse management actions to reduce nutrient inputs to surface waters, and stronger policies for rigorous, routine water quality data collection, as

well as establishment and enforcement of water quality standards. In Canada, there is also a need for such policies at the national level, to achieve consistency in standards and compliance across the country.

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Appendices

Appendix A. Model selection results predicting anuran responses in agricultural landscapes, based on four landscape predictors.

A.1. Delta AICc and associated measures from all possible models predicting anuran species richness in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	ΔAIC_c	Weight	LogLik	R^2
Crop diversity + Forest	3	0	0.29	-55.58	0.43
Forest	2	1.14	0.16	-57.35	0.29
Mean field size + Forest	3	1.83	0.12	-56.49	0.36
Crop diversity + Forest + Soybean	4	2.51	0.08	-55.54	0.43
Crop diversity + Mean field size + Forest	4	2.58	0.08	-55.58	0.43
Forest + Soybean	3	3.14	0.06	-57.14	0.30
Mean field size	2	3.51	0.05	-58.53	0.18
Mean field size + Forest + Soybean	4	4.36	0.03	-56.46	0.36
Crop diversity	2	4.71	0.03	-59.13	0.13
Crop diversity + Mean field size + Forest + Soybean	5	5.25	0.02	-55.53	0.43
Crop diversity + Mean field size	3	5.55	0.02	-58.35	0.20
NULL	1	5.59	0.02	-60.71	NA
Mean field size + Soybean	3	5.81	0.02	-58.48	0.19
Soybean	2	6.67	0.01	-60.11	0.05
Crop diversity + Soybean	3	7.01	0.01	-59.08	0.14
Crop diversity + Mean field size + Soybean	4	7.98	0.01	-58.27	0.21

A.2. Delta QAICc and associated measures from all possible models predicting anuran total abundance in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	$\Delta QAIC_c$	Weight	QLogLik	R^2
Mean field size + Forest	4	0	0.32	-36.78	0.34
Crop diversity + Forest	4	0.77	0.22	-37.16	0.43
Crop diversity + Mean field size + Forest	5	1.41	0.16	-36.10	0.40
Crop diversity + Forest + Soybean	5	2.10	0.11	-36.44	0.39
Mean field size + Forest + Soybean	5	2.50	0.09	-36.65	0.34
Crop diversity + Mean field size + Forest + Soybean	6	4.10	0.04	-35.96	0.39
Forest + Soybean	4	4.31	0.04	-38.93	0.28
Mean field size	3	6.25	0.01	-41.19	0.18
Forest	3	7.27	0.01	-41.70	0.27
Mean field size + Soybean	4	8.74	0.00	-41.15	0.18
Crop diversity + Mean field size	4	8.81	0.00	-41.18	0.19
Crop diversity + Mean field size + Soybean	5	11.49	0.00	-41.14	0.18
Crop diversity + Soybean	4	15.29	0.00	-44.42	0.11
Soybean	3	15.47	0.00	-45.80	0.05
Crop diversity	3	16.77	0.00	-46.45	0.13
NULL	2	21.93	0.00	-50.24	NA

A.3. Delta AICc and associated measures from all possible models predicting American toad occupancy in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	ΔAIC_c	Weight	LogLik	R^2
Crop diversity + Forest + Soybean	4	0	0.36	-5.01	0.35
Crop diversity + Forest	3	0.96	0.22	-6.78	0.28
Crop diversity + Mean field size + Forest + Soybean	5	2.37	0.12	-4.82	0.36
Crop diversity + Mean field size + Forest	4	2.53	0.10	-6.28	0.30
Crop diversity	2	2.83	0.09	-8.92	0.19
Crop diversity + Soybean	3	4.18	0.04	-8.39	0.22
Crop diversity + Mean field size	3	5.19	0.03	-8.89	0.19
Forest	2	6.51	0.01	-10.76	0.09
Crop diversity + Mean field size + Soybean	4	6.71	0.01	-8.37	0.23
NULL	1	7.35	0.01	-12.32	NA
Mean field size	2	7.96	0.01	-11.49	0.05
Mean field size + Forest	3	8.34	0.01	-10.47	0.10
Forest + Soybean	3	8.68	0.01	-10.62	0.09
Mean field size + Soybean	3	9.32	0.00	-10.96	0.09
Soybean	2	9.61	0.00	-12.31	<0.01
Mean field size + Forest + Soybean	4	9.79	0.00	-9.91	0.13

A.4. Delta AICc and associated measures from all possible models predicting green frog occupancy in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	ΔAIC_c	Weight	LogLik	R^2
Forest	2	0	0.27	-15.12	0.12
Crop diversity + Mean field size + Forest	4	1.76	0.11	-13.51	0.17
Crop diversity + Forest	3	1.85	0.11	-14.84	0.13
NULL	1	2.07	0.10	-17.29	NA
Mean field size + Forest	3	2.15	0.09	-14.99	0.13
Forest + Soybean	3	2.41	0.08	-15.12	0.12
Crop diversity	2	4.02	0.04	-17.14	0.01
Soybean	2	4.24	0.03	-17.24	<0.01
Crop diversity + Mean field size + Forest + Soybean	5	4.24	0.03	-13.37	0.17
Crop diversity + Forest + Soybean	4	4.27	0.03	-14.76	0.13
Mean field size	2	4.29	0.03	-17.27	<0.01
Mean field size + Forest + Soybean	4	4.54	0.03	-14.90	0.13
Crop diversity + Mean field size	3	6.40	0.01	-17.12	0.01
Crop diversity + Soybean	3	6.43	0.01	-17.13	0.01
Mean field size + Soybean	3	6.65	0.01	-17.24	<0.01
Crop diversity + Mean field size + Forest	4	8.95	0.00	-17.10	0.01

A.5. Delta AICc and associated measures from all possible models predicting spring peeper occupancy in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	ΔAIC_c	Weight	LogLik	R^2
Forest	2	0	0.33	-9.79	0.14
Crop diversity + Forest	3	1.87	0.13	-9.52	0.15
Mean field size + Forest	3	2.40	0.10	-9.79	0.14
Forest + Soybean	3	2.41	0.10	-9.79	0.14
NULL	1	2.79	0.08	-12.32	NA
Crop diversity + Mean field size + Forest	4	3.99	0.05	-9.29	0.17
Crop diversity + Forest + Soybean	4	4.32	0.04	-9.45	0.15
Crop diversity	2	4.49	0.04	-12.04	0.02
Soybean	2	4.59	0.03	-12.09	0.01
Mean field size	2	4.61	0.03	-12.10	0.01
Mean field size + Forest + Soybean	4	4.97	0.03	-9.78	0.14
Crop diversity + Mean field size + Forest + Soybean	5	6.73	0.01	-9.28	0.17
Crop diversity + Soybean	3	6.77	0.01	-11.97	0.02
Crop diversity + Mean field size	3	6.84	0.01	-12.01	0.02
Mean field size + Soybean	3	6.91	0.01	-12.04	0.02
Crop diversity + Mean field size + Soybean	4	9.34	0.00	-11.96	0.02

A.6. Delta AICc and associated measures from all possible models predicting wood frog occupancy in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	ΔAIC_c	Weight	LogLik	R^2
Forest	2	0	0.24	-12.24	0.11
Mean field size + Forest	3	1.54	0.11	-11.80	0.12
NULL	1	1.66	0.10	-14.20	NA
Crop diversity + Forest	3	1.93	0.09	-11.99	0.11
Mean field size	2	1.96	0.09	-13.22	0.05
Forest + Soybean	3	2.40	0.07	-12.23	0.11
Mean field size + Forest + Soybean	4	3.27	0.05	-11.37	0.14
Mean field size + Soybean	3	3.33	0.05	-12.69	0.08
Crop diversity	2	3.73	0.04	-14.10	0.01
Soybean	2	3.90	0.03	-14.19	<0.01
Crop diversity + Mean field size	3	4.07	0.03	-13.07	0.06
Crop diversity + Mean field size + Forest	4	4.12	0.03	-11.80	0.12
Crop diversity + Forest + Soybean	4	4.24	0.03	-11.86	0.12
Crop diversity + Mean field size + Soybean	4	5.74	0.01	-12.61	0.08
Crop diversity + Mean field size + Forest + Soybean	5	6.02	0.01	-11.37	0.14
Crop diversity + Soybean	3	6.14	0.01	-14.10	0.01

A.7. Delta AICc and associated measures from all possible models predicting gray tree frog occupancy in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	ΔAIC_c	Weight	LogLik	R^2
Mean field size	2	0	0.16	-21.87	0.07
NULL	1	0.06	0.15	-23.03	NA
Forest	2	0.30	0.13	-22.02	0.06
Crop diversity	2	1.25	0.08	-22.50	0.03
Mean field size + Forest	3	1.48	0.08	-21.40	0.09
Crop diversity + Forest	3	1.53	0.07	-21.43	0.09
Mean field size + Soybean	3	1.83	0.06	-21.58	0.08
Soybean	2	2.17	0.05	-22.95	0.01
Crop diversity + Mean field size	3	2.40	0.05	-21.86	0.07
Forest + Soybean	3	2.70	0.04	-22.01	0.06
Mean field size + Forest + Soybean	4	3.49	0.03	-21.12	0.11
Crop diversity + Soybean	3	3.65	0.03	-22.49	0.03
Crop diversity + Mean field size + Forest	4	3.85	0.02	-21.30	0.10
Crop diversity + Forest + Soybean	4	3.93	0.02	-21.34	0.10
Crop diversity + Mean field size + Soybean	4	4.37	0.02	-21.56	0.08
Crop diversity + Mean field size + Forest + Soybean	5	5.97	0.01	-20.98	0.11

A.8. Delta AICc and associated measures from all possible models predicting leopard frog occupancy in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

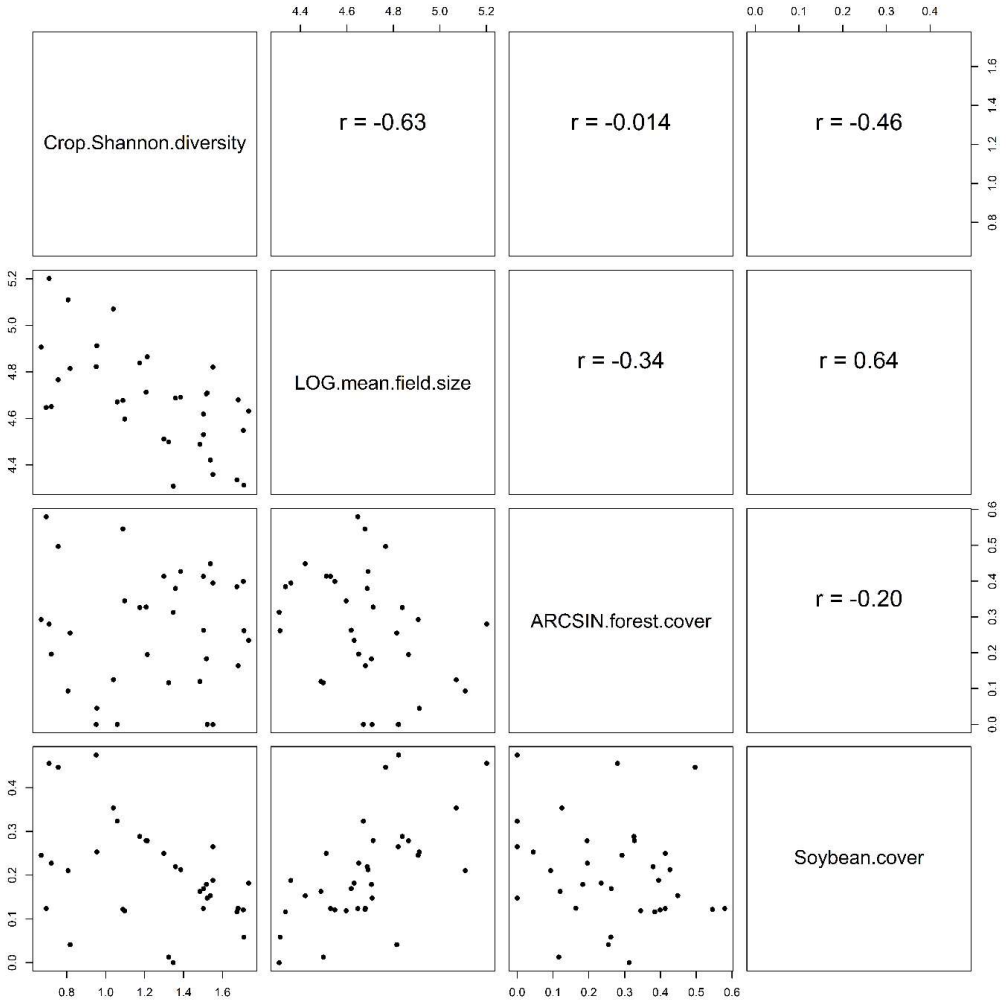
Model	K	ΔAIC_c	Weight	LogLik	R^2
Crop diversity	2	0	0.22	-17.44	0.12
Mean field size	2	0.80	0.14	-17.84	0.10
Crop diversity + Mean field size	3	1.78	0.09	-17.12	0.13
Crop diversity + Soybean	3	1.79	0.09	-17.13	0.13
Soybean	2	1.94	0.08	-18.41	0.07
NULL	1	2.16	0.07	-19.65	NA
Crop diversity + Forest	3	2.25	0.07	-17.36	0.12
Mean field size + Soybean	3	2.98	0.05	-17.72	0.10
Mean field size + Forest	3	3.13	0.05	-17.8	0.10
Crop diversity + Mean field size + Soybean	4	4.19	0.03	-17.03	0.14
Forest	2	4.28	0.03	-19.58	0.00
Crop diversity + Forest +Soybean	4	4.30	0.03	-17.09	0.13
Forest + Soybean	3	4.34	0.03	-18.4	0.07
Crop diversity + Mean fields size + Forest	4	4.36	0.02	-17.12	0.13
Mean field size + Forest + Soybean	4	5.48	0.01	-17.68	0.11
Crop diversity + Mean field size + Forest + Soybean	5	6.94	0.01	-17.03	0.14

A.9. Delta AICc and associated measures from all possible models predicting western chorus frog occupancy in agricultural landscapes based on crop diversity, mean crop field size, and proportions of forest and soybean cover.

Model	K	ΔAIC_c	Weight	LogLik	R^2
Forest + Soybean	3	0	0.19	-8.19	0.17
Forest	2	0.14	0.18	-9.46	0.14
Mean field size + Forest	3	0.40	0.15	-8.39	0.17
Mean field size + Forest + soybean	4	2.03	0.07	-7.91	0.18
Crop diversity + Mean field size + Forest	4	2.14	0.06	-7.97	0.19
Crop diversity + Forest	3	2.41	0.06	-9.39	0.14
Crop diversity + Forest + Soybean	4	2.58	0.05	-8.19	0.17
Crop diversity + Mean field size	3	2.74	0.05	-9.56	0.14
Mean field size	2	2.82	0.05	-10.80	0.08
Soybean	2	3.22	0.04	-11.00	0.06
NULL	1	3.58	0.03	-12.32	NA
Crop diversity + Mean field size + Forest + Soybean	5	4.22	0.02	-7.63	0.20
Mean field size + Soybean	3	4.76	0.02	-10.57	0.09
Crop diversity + Mean field size + Soybean	4	5.01	0.02	-9.40	0.15
Crop diversity + Soybean	3	5.26	0.01	-10.80	0.08
Crop diversity	2	5.84	0.01	-12.31	<0.01

Appendix B. Pairwise correlations between Chapter 2 landscape predictor variables and variance inflation factors for each predictor in each global model for each anuran response.

B.1. Matrix scatterplot showing pairwise correlations between predictor variables.



B.2. Variance inflation factors (VIFs) for each predictor (crop diversity, mean crop field size, and proportions of forest and soybean cover) in each global model (model containing all four predictors) for each response (anuran species richness, total abundance, and presence/absence for each individual species).

Predictor / Model	Crop Shannon diversity	LOG Mean field size	ARCSIN Forest cover	Soybean cover
Species richness	1.93	2.79	1.30	1.77
Total abundance	1.86	2.68	1.26	1.66
American toad	1.42	1.28	1.36	1.29
Green frog	3.13	4.94	2.17	2.04
Spring peeper	1.61	1.8	1.3	1.62
Wood frog	2.17	2.53	1.21	1.79
Gray tree frog	1.76	2.52	1.22	1.88
Leopard frog	1.5	2.44	1.27	1.64
Chorus frog	2.31	2.65	1.24	1.37

Appendix C. Pairwise correlations between Chapter 3 predictor variables in the path model and variance inflation factors of each predictor in each global model for each water quality response.

C.1. Pairwise correlations of all the predictor variables in the path model (Figure 3.2).

	Forest	**Crop**	**Edge**	**Nitrogen**	**Pesticide**
Crop	-0.55	-	-	-	-
Edge	-0.02	-0.48	-	-	-
Nitrogen	-0.22	0.43	-0.16	-	-
Pesticide	-0.44	0.23	-0.11	-0.09	-
Oxygen	-0.17	0.25	-0.07	-0.31	-0.16

C.2. Variance inflation factors (VIF) of each predictor in each global model (model containing all hypothesized predictors for a given response; Figure 3.2) for each water quality response.

	Predictor					
Model response variable	**Forest**	**Crop**	**Edge**	**Nitrogen**	**Pesticide**	**Oxygen**
Nitrogen	1.70	2.21	1.53			
Pesticide	1.70	2.21	1.53			
Invertebrate richness	1.66		1.11	1.22	1.55	1.36
Decomposition				1.15	1.07	1.19
***C. dubia* survival**					1.02	1.02
***C. dubia* reproduction**					1.01	1.01

Appendix D. Analytical methods and quality control information for determination of pesticide concentrations in ditch water samples.

D.1.1. Method of analysis for determination of atrazine concentrations in water samples from farmland drainage ditch sites.

Solid-phase extraction (SPE) procedure for atrazine concentration:

Water samples (100 ml aliquot) were spiked with a mixed internal standard (IS) solution, stirred for a few minutes and passed through a Supelclean SPE cartridge, which had been sequentially pre-conditioned with 10 ml methanol and 10 ml water. The analyte (and IS) were eluted with 4 ml of methanol. An aliquot of 200 µL of the eluate was diluted with an equal volume of water. The diluted extract was filtered through Millex® HV 4 mm syringe filter - 0.45 µm PVDF membrane and transferred in a 2 mL auto-sampler vial containing a 500 µL glass insert.

High-performance liquid chromatography analysis for determination of atrazine in water samples:

Samples of 20 µL aliquots at 20°C were injected into the high-performance liquid chromatograph (Agilent 1200 Series) and separated with an Atlantis ® dC18 column (3-µm diameter particle size, 2.1 mm x 50 mm; WATERS) at 35°C with a flow rate of 600 µL/ min. The 70:30 isocratic mobile phase consisted of methanol containing 0.05% formic acid and 10 mM ammonium acetate also containing 0.05% formic acid. Run time was 5 minuets.

Triple quadropole mass spectrometer operating conditions for determination of atrazine in water samples:

Atrazine was detected by mass spectrometry using AB Sciex API 5000 Triple Quadropole Mass Spectrometer with the TurboSpray ion source in positive polarity using multiple reaction monitoring (MRM). Two MRM transitions were monitored. The MRM parameters and mass spectrometer settings are given in Tables S1 and S2.

Quality assurance for atrazine analyses:

The analytical and internal standards for atrazine analysis were purchased from Caledon, ChemService and had chemical purities ≥98%. Ammonium acetate, methanol, and formic acid were HPLC grade. The stock solution was diluted in methanol:water 1:1 to prepare the calibration standards. A d5-Atrazine (ethyl-D5) from CDN Isotopes standard was used to spike the samples. A second source Atrazine standard solution was purchase from Supelco. The calibration curve was built with 7 levels of concentrations, ranging from 0.005 to 1.0 pg/µL (or ppb) with R > 0.995 (peak area; linear regression; no weighting). A calibration curve was prepared on each day of injection. Sample concentrations were calculated using the internal standard method. The water samples (100 ml) concentrated on SPE, were spiked with 25 µL of 320 pg/µL IS spiking solution (d5-Atrazine). Two to three blanks (H2O:MeOH, 50:50) were injected at the beginning and the end of each set of samples and before and after the calibration standards to monitor injection cross-contamination. A sample blank (100 mL of RO water spiked with IS) was extracted/concentrated on a Supelclean SPE cartridge and analyzed with each set of 10 samples to monitor contamination from the process. One random sample per set of 10 samples was extracted/concentrated by SPE in duplicate and injected (also in duplicate) on the same day to determine precision. Aliquots of various levels of calibration standards were injected between each set of 10-15 samples. The verification standards

were all within 10% of the expected concentrations. To monitor accuracy, an aliquot of 1.0 ppb solution (prepared commercially - 2nd source STD) was injected and analyzed along with the samples to monitor day to day variation. The limit of detection was determined by spiking a blank matrix (post SPE extract, concentrated 12.5x and containing no or only traces of Atrazine) with a pure (98%) ChemService standard solution. It was confirmed that the analysis could detect as low as 0.005 ppb with a signal to noise ratio of at least 3 for a 20 uL injection, meaning that the initial sample solution concentration could be as low as 0.0004 ppb (12.5x concentration factor). The limits of quantification (5 times signal to noise ratio) post-SPE were evaluated to be 0.002 µg/L

Method validation for atrazine analyses:

No atrazine was found in the blanks (solvent blanks or SPE sample blanks) and there was no cross-contamination between injections or during the SPE process. The percent difference between results for the 6 water samples extracted/concentrated by SPE in duplicates were all 4% or lower demonstrating excellent precision in the procedure. The recoveries of the verification standards of various concentrations were all between 90-110% demonstrating good accuracy and stability from start to end of a daily injection sequence (up to 6 hours).

D.1.2. Multiple reaction monitoring (MRM) transitions and mass spectrometer parameters used for the analysis of atrazine and the internal standard (IS).

Compound	**Parent ion (Da)**	**Daughter ion (Da)**	**Collision energy (CE)(V)**	**Cell exit potential (CXP)(V)**	**Dwell Time (msec)**
Atrazine	215	174	25	12	150
	215	104	41	18	150

Atrazine –d5	220	179	27	14	150
	220	101	35	16	150

D.1.3. Triple quadropole mass spectrometer settings for the analysis of atrazine.

Parameter	Setting
Curtain gas	20
Gas 1	70
Gas 2	70
Ion Spray voltage	4000 V
Temperature	600 °C
Interface heater	ON
Collision gas	8
Delustering potential	86 V
Entrance potential	10 V
Channel electron multiplier	2000
Deflector	0

D.2.1. Method of analysis for determination of glyphosate concentrations in water samples from farmland drainage ditch sites.

Solid-phase extraction (SPE) procedure for glyphosate concentration:

Water samples (25 ml aliquot) were spiked with the IS, stirred for a few minutes and passed through an Oasis MAX anion exchange cartridge (6 cc, 500 mg), which had been sequentially pre-conditioned with 10 ml methanol and 10 ml water. An aliquot of 25 ml of sample was loaded on the cartridge. The cartridge was then sequentially rinsed with: 10 ml of 5% NH4OH, 10 ml of water and finally, 10 ml of methanol. The analyte (and IS) were eluted with 10 mL 5% formic acid in methanol. The eluate was evaporated to dryness and reconstituted in 1 ml of ammonium bicarbonate buffer and filtered through

Millex® HV 4 mm syringe filter - 0.45 µm PVDF membrane and transferred in a 2 mL auto-sampler vial.

High-performance liquid chromatography analysis for determination of glyphosate in water samples:

Samples of 50 µL aliquots at 20°C were injected into the high-performance liquid chromatograph (Agilent 1200 Series) and separated with an IC-Pak Anion HR column (6-µm diameter particle size, 4.6 mm x 75 mm; WATERS) at 35°C with a flow rate of 750 µL/ min. The isocratic mobile phase consisted of 100mM ammonium bicarbonate pH 8.0. Run time was 3 minuets.

Triple quadropole mass spectrometer operating conditions for determination of glyphosate in water samples:

Glyphosate was detected by mass spectrometry using AB Sciex API 5000 Triple Quadropole Mass Spectrometer with the TurboSpray ion source in negative polarity using MRM (multiple reaction monitoring) scan type. Two MRM transitions were monitored. The MRM parameters and mass spectrometer settings are given in Tables S3 and S4.

Quality assurance for glyphosate analyses:

The analytical and internal standards for glyphosate analysis were purchased from Caledon, ChemService and had chemical purities ≥99%. The stock solution was diluted in RO water and in ammonium bicarbonate buffer to prepare the calibration standards. A 2-C13, N15;-Glyphosate from CIL (CNLM-4666-S; lot SCEF-001) was used to spike the samples concentrated by SPE. A second source Glyphosate standard solution was

purchase from Supelco (98.6% purity; cat# 44690-U; lot# LC05403). The calibration curve was built with 7 levels of concentrations, ranging from 1.0 to 100 pg/µL (or ppb) with R > 0.995 (peak area; linear regression through zero; no weighting). A calibration curve was prepared on each day of injection. Sample concentrations were calculated using the internal standard method. The water samples (25 ml) concentrated on SPE, were spiked with 50 µL of 1.0 ng/µL IS spiking solution. Two to three blanks (reverse osmosis water) were injected at the beginning and the end of each set of samples and before and after the calibration standards to monitor injection cross-contamination. A sample blank (25 mL of reverse osmosis water spiked with IS) was extracted/concentrated on an Oasis MAX anion exchange cartridge and analyzed with each set of 10 samples to monitor contamination from the process. Random post-SPE extracts were injected in duplicates or triplicates in the same sequence or on different days. One random sample per set of 10 samples was extracted/concentrated by SPE in duplicate. Some SPE were repeated on a different day to determine precision. Aliquots of various levels of calibration standards were injected between each set of 10-15 samples and the recoveries were monitored. To monitor accuracy, an aliquot of 50 ppb solution (prepared commercially - 2nd source STD) was injected and analyzed along with the samples to monitor day to day variation. The limit of detection was determined by spiking a blank matrix (post SPE extract, concentrated 25x and containing no Glyphosate) with a pure (99.5%) ChemService Glyphosate standard solution. It was confirmed that the analysis could detect as low as 0.65 ppb with a signal to noise ratio of at least 3 for a 50 uL injection, meaning that the initial sample solution concentration

could be as low as 0.025 ppb (for 25x concentration factor). The limits of quantification (3 times signal to noise ratio) post-SPE were evaluated to be 0.08 µg/L.

Method validation for glyphosate analyses:

No glyphosate was found in the blanks (solvent blanks or SPE sample blanks) and there was no cross-contamination between injections or during the SPE process. The percent difference between results for the water samples extracted/concentrated by SPE in duplicates (even on a different day) were 13% or lower demonstrating good precision. The recoveries of the verification standards of various concentrations were all between 85-115% demonstrating good accuracy and stability from start to end of a daily injection sequence (up to 6 hours). The methods were demonstrated to be accurate: the recoveries for the QC sample (second source Glyphosate) ranged from 90% to 105%, with an average of 97% and a %CV of 3.8% for a total of 22 replicates on 10 days of injection.

D.2.2. Multiple reaction monitoring (MRM) transitions and mass spectrometer parameters used for the analysis of glyphosate and the internal standard (IS).

Compound	**Precursor ion (Da)**	**Product ion (Da)**	**Collision energy (CE)(V)**	**Cell exit potential (CXP)(V)**	**Dwell Time (msec)**
Glyphosate	168.3	62.9	-30	-9	200
	168.3	79.0	-56	-13	200
Glyphosate –d2	170.4	62.9	-30	-9	200
	170.4	79.0	-56	-13	200

D.2.3. Triple quadropole mass spectrometer settings for the analysis of glyphosate.

Parameter	**Setting**
Curtain gas	10

Gas 1	70
Gas 2	70
Ion Spray voltage	-4500 V
Temperature	750 °C
Interface heater	ON
Collision gas	8
Delustering potential	-60 V
Entrance potential	-2.0 V
Channel electron multiplier	2000
Deflector	0

D.3.1. Method of analysis for determination of the concentrations of four neonicotinoids—Acetamiprid, Clothianidin, Imidacloprid and Thiamethoxam—in water samples from farmland drainage ditch sites.

Solid-phase extraction (SPE) procedure for neonicotinoid concentration:

Water samples (100 ml aliquot) were spiked with the mixed internal standard solution (IS), stirred for a few minutes and passed through an Oasis HLB cartridge, which had been sequentially pre-conditioned with 10 ml methanol and 10 ml water. The analytes (and IS) were eluted with 10 ml of methanol. The eluates were evaporated to dryness and the extracts were re-dissolved in 500 µL of water: acetonitrile 80:20. The re-dissolved sample was filtered through Millex® HV 4 mm syringe filter - 0.45 µm PVDF membrane and transferred in a 2mL auto-sampler vial containing a 500 µL flat bottom glass insert.

High-performance liquid chromatography analysis for determination of four neonicotinoids in water samples:

Samples of 10 µL aliquots at 20°C were injected into the high-performance liquid chromatograph (Agilent 1200 Series) and separated with an X-Terra® MS C8 column (3.5-µm diameter particle size, 2.1 mm x 100 mm; WATERS) at 40°C with a flow rate of

0.5 mL/min. The 80:20 isocratic mobile phase consisted of reverse osmosis (RO) water with 0.1% formic acid and acetonitrile with 0.1% formic acid.

Triple quadropole mass spectrometer operating conditions for determination of four neonicotinoids in water samples:

Acetamiprid, Clothianidin, Imidacloprid, and Thiamethoxam were detected by mass spectrometry using AB Sciex API 5000 Triple Quadropole Mass Spectrometer with the TurboSpray ion source in positive polarity using multiple reaction monitoring (MRM). The MRM parameters and mass spectrometer settings are given in Tables S5 and S6.

Quality assurance for neonicotinoid analyses:

The analytical and internal standards for neonicotinoid analyses were obtained from Sigma-Aldrich and had chemical purities ≥98%. Acetonitrile, methanol, and formic acid were HPLC grade. The calibration curve was built with 8 levels of concentrations, ranging from 0.10 to 20 pg/µL with R > 0.995 (linear regression, no weighting). A calibration curve was prepared on each day of injection. Sample concentrations were calculated using the internal standard method. Two to three blanks (H2O:ACN 80:20) were injected at the beginning and the end of each set of samples and before and after the calibration standards to monitor injection cross-contamination. A sample blank (100 mL of RO water spiked with four IS) was extracted/concentrated on an Oasis SPE cartridge and analyzed with each set of 10-12 samples to monitor possible contamination from the process. Precision was assessed by concentrating one random sample per set (10 to 12 samples) on an Oasis SPE in duplicate and injected (also in duplicate) on the same day. Standards of different levels were analyzed with each set of 10 to12 samples and the

percentage of variation between the experimental and expected values were calculated. To monitor accuracy, aliquot of 20 ppb Imidacloprid solution (prepared commercially - 2nd source STD) was injected and analyzed along with the samples to monitor day to day variation. The recoveries were evaluated in triplicates and corrected for their respective IS recovery at 3 different concentrations for each of the 4 neonicotinoids. The limits of detection (3 times signal to noise ratio) post-SPE (X200 concentration) were determined to be 0.0001 µg/L for acetamiprid, 0.00025 µg/L for clothianidin and imidacloprid, and 0.0002 µg/L for thiamethoxam. The limits of quantification (10 times signal to noise ratio) post-SPE were evaluated to be 0.0003 µg/L for acetamiprid, 0.00075 µg/L for clothianidin and imidacloprid, and 0.0006 µg/L for thiamethoxam.

Method validation for neonicotinoid analyses:

Six random samples were extracted and injected in duplicates on the same day, and eight samples were repeated on a different day. The percent difference between duplicates ranged between 0% and 16%, with most values under 5% demonstrating excellent precision in the procedure. The recoveries for the verification standards of various concentrations were all between 90-110% demonstrating good accuracy and stability from start to end of a daily injection sequence (up to 12 hours). The method was also demonstrated to be accurate: the recoveries for the QC sample (second source Imidacloprid) ranged from 94% to 99%, with an average of 97.5% and % CV of 1.6%. No carry-over was observed in solvent blanks.

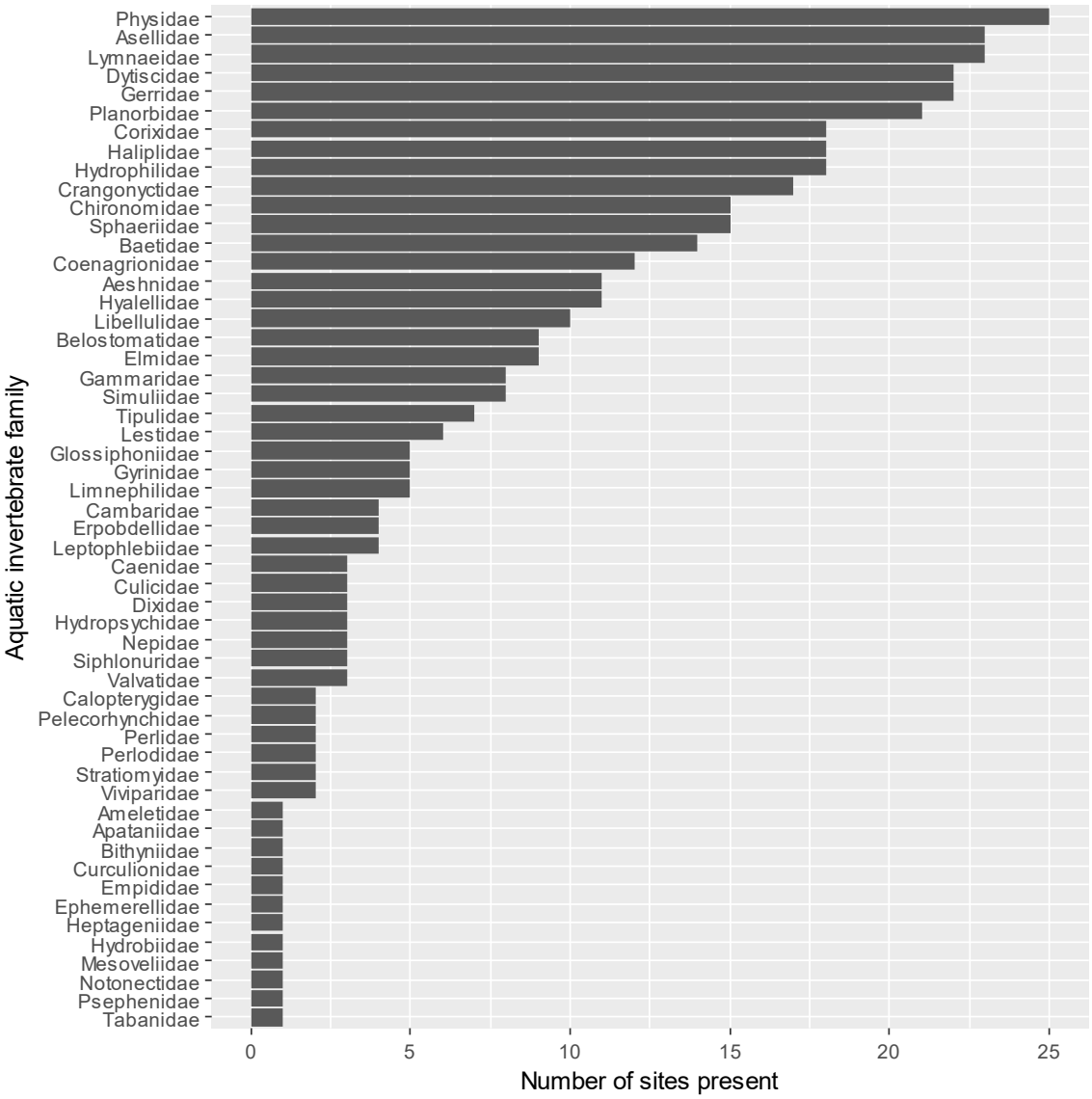
D.3.2. Multiple reaction monitoring (MRM) transitions and mass spectrometer parameters used for the analysis of four neonicotinoid insecticides and their internal standards (IS).

Neonicotinoids	Parent ion (Da)	Daughter ion (Da)	De-clustering potential (DP) (V)	Collision energy (CE) (V)	Cell exit potential (CXP) (V)	Dwell Time (msec)
Acetamiprid	222.4	126.0	96	29	22	150
Acetamirpid-d3 (IS)	225.4	126.0	96	29	22	150
Clothianidin	249.5	169.0	66	19	22	150
Clothianidin-d3 (IS)	252.5	172.0	66	19	22	150
Imidacloprid	255.6	209.1	96	21	28	150
Imidacloprid-d4 (IS)	259.6	213.1	96	21	28	150
Thiamethoxam	291.7	211.1	76	19	18	150
Thiamethoxam-d3	294.7	214.1	76	19	18	150

D.3.3. Triple quadropole mass spectrometer settings for the analysis of four neonicotinoid insecticides.

Parameter	Setting
Curtain gas	50
Gas 1	70
Gas 2	40
Ion Spray voltage	3000 V
Temperature	750 °C
Interface heater	ON
Collision gas	7
Entrance potential	10 V
Channel electron multiplier	2000
Deflector	0

Appendix E. The 54 aquatic macroinvertebrate families found in samples collected from 27 agricultural drainage ditches in Eastern Ontario, Canada, and the number of sites in which each family was encountered, in decreasing order.



Appendix F. Principal components analyses (PCA) with axis loadings, eigenvalues, and % variance explained, for three pesticide variables (F.1), and two nitrogen variables (F.2).

F.1. Principal components analysis (PCA) with axis loadings, eigenvalues, and % variance explained for three pesticide variables (square-root transformed mean atrazine, square-root transformed mean glyphosate, and square-root transformed mean neonicotinoids) measured in water samples collected from 27 farm drainage ditches in Eastern Ontario.

Original variable	**PC1**	**PC2**	**PC3**
Mean Atrazine	0.85	-0.19	-0.50
Mean Glyphosate	0.80	-0.40	0.44
Mean Neonicotinoids	0.61	0.78	0.11
Eigenvalue	1.74	0.81	0.45
% Variance	58.02	26.91	15.07

F.2. Principal components analysis (PCA) with axis loadings, eigenvalues, and % variance explained for two inorganic nitrogen variables (square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen) measured in water samples collected from 27 farm drainage ditches in Eastern Ontario.

Original variable	**PC1**	**PC2**
Total ammonia	0.79	-0.62
Nitrate-nitrogen + Nitrite-nitrogen	0.79	0.62
Eigenvalue	1.23	0.77
% Variance	61.68	38.32

Appendix G. All conditionally independent pairs of variables, structured as independence claims, implied by the path model of the predicted relationships between landscape predictors and drainage ditch water quality response variables (Figure 3.2), and the associated models constructed to test the independence claims. Also shown are the null probabilities (*p* values) used to calculate Fisher's C statistic to test the correlational structure of the full path model. The independence claim notation identifies the tested pair of variables in parentheses, followed by the variables that were statistically controlled for while testing the independence between the pair, in curled brackets.

Independence claim	**Model** [a]	***p* value**
(FOR, ED) {CR}	FOR ~ ED + CR	0.05
(FOR, DO) {CR,N}	FOR ~ DO + CR + N	0.87
(FOR, DC) {CR,N,PT,DO}	FOR ~ DC + CR + N + PT + DO	0.17
(SV, FOR) {CR,N,PT}	SV ~ FOR + CR + N + PT	0.67
(FOR, RR) {CR,N,PT}	FOR ~ RR + CR + N + PT	0.66
(CR, DO) {N}	CR ~ DO + N	0.02
(IR, CR) {FOR,ED,N,PT,DO}	IR ~ CR + FOR + ED + N + PT + DO	0.11
(CR, DC) {N,PT,DO}	CR ~ DC + N + PT + DO	0.56
(SV, CR) {N,PT}	SV ~ CR + N + PT	0.88
(CR, RR) {N,PT}	CR ~ RR + N + PT	0.30
(ED, DO) {CR,N}	ED ~ DO + CR + N	0.61
(ED, DC) {CR,N,PT,DO}	ED ~ DC + CR + N + PT + DO	0.40
(SV, ED) {CR,N,PT}	SV ~ ED + CR + N + PT	0.83
(ED, RR) {CR,N,PT}	ED ~ RR + CR + N + PT	0.75
(N, PT) {FOR,CR,ED}	N ~ PT + FOR + CR + ED	0.33
(PT, DO) {FOR,CR,ED,N}	PT ~ DO + FOR + CR + ED + N	0.04
(SV, DO) {N,PT}	SV ~ DO + N + PT	0.54
(DO, RR) {N,PT}	DO ~ RR + N + PT	0.58
(IR, DC) {FOR,ED,N,PT,DO}	IR ~ DC + FOR + ED + N + PT + DO	0.84
(IR, SV) {FOR,ED,N,PT,DO}	IR ~ SV + FOR + ED + N + PT + DO	0.78
(IR, RR) {FOR,ED,N,PT,DO}	IR ~ RR + FOR + ED + N + PT + DO	0.90
(SV, DC) {N,PT,DO}	SV ~ DC + N + PT + DO	0.95
(DC, RR) {N,PT,DO}	DC ~ RR + N + PT + DO	0.59
(SV, RR) {N,PT}	SV ~ RR + N + PT	0.46

Notes: FOR = forest cover, CR = high-intensity crop cover, ED = edge cover, N = inorganic nitrogen, PT = pesticides, DO = dissolved oxygen, IR = aquatic macroinvertebrate richness, DC = decomposition, SV = *C. dubia* survival, RR = *C. dubia*

reproductive rate. [a] Relationships with the response variables IR and SV were tested using generalized linear models with a Poisson distribution and log-link. Relationships between all other variables were tested using linear models.

Appendix H. Estimates of model coefficient(s), AIC_c and associated measures, and R^2 values for each Chapter 3 response variable modelled on its candidate set of standardized predictors.

H.1. Proportion of the landscape in forest cover (square-root transformed and standardized) modelled on the proportion of the landscape in high-intensity crop cover (arcsine transformed and standardized); n=27.

Model	Crop estimate	K	AICc	ΔAICc	ModLik	AICcWt	R^2
Crop	-0.55	3	72.81	0.00	1.00	0.97	0.31
null		2	80.10	7.29	0.03	0.03	NA

H.2. Proportion of the landscape in field edge cover modelled on the proportion of the landscape in high-intensity crop cover (arcsine transformed and standardized); n=27.

Model	Crop estimate	K	AICc	ΔAICc	ModLik	AICcWt	R^2
Crop	-0.48	3	75.55	0.00	1.00	0.91	0.23
null		2	80.10	4.55	0.10	0.09	NA

H.3. Pesticides (linear combination of square-root transformed mean atrazine, square-root transformed mean glyphosate, and square-root transformed summed mean clothianidin, imidacloprid, and thiamethoxam) in ditches modelled on the standardized proportions of square-root transformed forest cover, arcsine transformed high-intensity crop cover, and field edge cover in the surrounding 1-km radius agricultural landscapes; n=27.

Model	Forest estimate	Crop estimate	Edge estimate	K	AICc	ΔAICc	ModLik	AICcWt	R^2
Forest	-0.44			3	76.70	0.00	1.00	0.49	0.20
Forest + Edge	-0.45		-0.12	4	78.97	2.27	0.32	0.16	0.21
Forest + Crop	-0.46	-0.03		4	79.46	2.75	0.25	0.12	0.20
null				2	80.10	3.40	0.18	0.09	NA

Crop		0.23		3	81.24	4.54	0.10	0.05	0.05
Global	-0.55	-0.18	-0.21	5	81.51	4.81	0.09	0.04	0.23
Edge			-0.11	3	82.30	5.59	0.06	0.03	0.01
Crop + Edge		0.22	-0.01	4	84.02	7.32	0.03	0.01	0.05

H.4. Inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen) in ditches modelled on the standardized proportions of square-root transformed forest cover, arcsine transformed high-intensity crop cover, and field edge cover in the surrounding 1-km radius agricultural landscapes; n=27.

Model	Forest	Crop	Edge	K	AICc	ΔAICc	ModLik	AICcWt	R^2
Crop		0.43		3	77.17	0.00	1.00	0.48	0.18
Crop + Edge		0.46	0.06	4	79.86	2.68	0.26	0.13	0.19
Forest + Crop	0.03	0.45		4	79.93	2.75	0.25	0.12	0.18
null				2	80.10	2.93	0.23	0.11	NA
Forest	-0.22			3	81.36	4.19	0.12	0.06	0.05
Edge			-0.16	3	81.95	4.77	0.09	0.04	0.03
Global	0.06	0.50	0.08	5	82.82	5.64	0.06	0.03	0.19
Forest + Edge	-0.22		-0.16	4	83.36	6.19	0.05	0.02	0.07

H.5. Standardized mean dissolved oxygen (mg/L) modelled on inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen) in ditches; n=27.

Model	Nitrogen estimate	K	AICc	ΔAICc	ModLik	AICcWt	R^2
Nitrogen	-0.31	3	79.85	0.00	1.00	0.53	0.10
null		2	80.10	0.25	0.88	0.47	NA

H.6. Aquatic macroinvertebrate family richness modelled on the standardized proportions of square-root transformed forest cover and field edge cover in surrounding 1-km radius

agricultural landscapes, and inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen), pesticides (a linear combination of square-root transformed mean atrazine, square-root transformed mean glyphosate, and square-root transformed summed mean clothianidin, imidacloprid, and thiamethoxam), and standardized mean dissolved oxygen (mg/L) in ditches; n=27.

Model	Forest estimate	Edge estimate	Nitrogen estimate	Pesticide estimate	Oxygen estimate	K	AICc	ΔAICc	ModLik	AICcWt	R^2
Nitrogen + Oxygen			-0.12		0.10	3	150.17	0.00	1.00	0.21	0.36
Nitrogen			-0.15			2	151.63	1.46	0.48	0.10	0.23
Edge + Nitrogen + Oxygen		0.05	-0.11		0.11	4	151.79	1.62	0.45	0.09	0.39
Oxygen					0.14	2	151.95	1.78	0.41	0.09	0.24
Edge + Oxygen		0.07			0.14	3	152.63	2.46	0.29	0.06	0.29
Nitrogen + Pesticide + Oxygen			-0.12	-0.02	0.10	4	152.81	2.64	0.27	0.06	0.36
Forest + Nitrogen + Oxygen	0.01		-0.11		0.11	4	152.87	2.70	0.26	0.06	0.36
Edge + Nitrogen		0.04	-0.14			3	153.60	3.43	0.18	0.04	0.25
Nitrogen + Pesticide			-0.15	-0.04		3	153.62	3.45	0.18	0.04	0.25
Forest + Oxygen	0.04				0.14	3	153.89	3.72	0.16	0.03	0.26
Forest + Nitrogen	-0.01		-0.15			3	154.11	3.94	0.14	0.03	0.24
Pesticide + Oxygen				-0.01	0.14	3	154.48	4.31	0.12	0.02	0.24
Forest + Edge + Oxygen	0.04	0.07			0.15	4	154.67	4.50	0.11	0.02	0.31
Forest + Edge + Nitrogen + Oxygen	0.02	0.06	-0.10		0.12	5	154.68	4.51	0.11	0.02	0.39
Edge + Nitrogen + Pesticide + Oxygen		0.05	-0.11	-0.01	0.11	5	154.79	4.62	0.10	0.02	0.39
Edge + Pesticide + Oxygen		0.07		0.00	0.14	4	155.41	5.24	0.07	0.02	0.29
Forest + Nitrogen + Pesticide + Oxygen	0.01		-0.12	-0.01	0.10	5	155.84	5.67	0.06	0.01	0.36
Forest + Nitrogen + Pesticide	-0.04		-0.16	-0.05		4	155.94	5.77	0.06	0.01	0.27
Edge + Nitrogen + Pesticide		0.03	-0.14	-0.03		4	155.95	5.78	0.06	0.01	0.27
Forest + Edge + Nitrogen	-0.01	0.04	-0.14			4	156.33	6.16	0.05	0.01	0.25
Forest + Pesticide + Oxygen	0.05			0.02	0.15	4	156.56	6.39	0.04	0.01	0.26
null						1	156.95	6.78	0.03	0.01	NA
Forest + Edge + Pesticide + Oxygen	0.06	0.07		0.03	0.16	5	157.39	7.22	0.03	0.01	0.32
Global	0.02	0.06	-0.10	0.00	0.12	6	158.02	7.85	0.02	0.00	0.39
Edge		0.06				2	158.02	7.85	0.02	0.00	0.04
Forest + Edge + Nitrogen + Pesticide	-0.03	0.03	-0.15	-0.05		5	158.63	8.46	0.01	0.00	0.28
Pesticide				-0.03		2	159.00	8.83	0.01	0.00	0.01
Forest	0.02					2	159.20	9.03	0.01	0.00	0.00
Edge + Pesticide		0.05		-0.02		3	160.40	10.23	0.01	0.00	0.05
Forest + Edge	0.02	0.06				3	160.45	10.28	0.01	0.00	0.04
Forest + Pesticide	0.00			-0.03		3	161.54	11.37	0.00	0.00	0.01
Forest + Edge + Pesticide	0.01	0.05		-0.02		4	163.15	12.98	0.00	0.00	0.05

H.7. Leaf litter decomposition, measured as the proportion of weight lost, modelled on inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen), pesticides (a linear combination of square-root transformed mean atrazine, square-root transformed mean glyphosate, and square-root transformed summed mean clothianidin, imidacloprid, and thiamethoxam), and standardized mean dissolved oxygen (mg/L) in ditches; n=25 (note that litter decomposition bags were retrieved from 25 of the 27 ditches).

Model	Nitrogen estimate	Pesticide estimate	Oxygen estimate	K	AICc	ΔAICc	ModLik	AICcWt	R^2
null				2	-3.24	0.00	1.00	0.48	NA
Pesticide		0.01		3	-0.77	2.48	0.29	0.14	0.00
Oxygen			0.01	3	-0.72	2.53	0.28	0.14	0.00
Nitrogen	0.01			3	-0.67	2.57	0.28	0.13	0.00
Pesticide + Oxygen		0.02	0.01	4	1.97	5.21	0.07	0.04	0.01
Nitrogen + Pesticide	0.01	0.01		4	2.06	5.30	0.07	0.03	0.01
Nitrogen + Oxygen	0.01		0.01	4	2.08	5.32	0.07	0.03	0.01
Global	0.01	0.02	0.02	5	5.02	8.26	0.02	0.01	0.01

H.8. *Ceriodaphnia dubia* survival in water collected from ditches, modelled on inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen) and pesticides (a linear combination of square-root transformed mean atrazine, square-root transformed mean glyphosate, and square-root transformed summed mean clothianidin, imidacloprid, and thiamethoxam) in ditch water; n=27.

Model	Nitrogen estimate	Pesticide estimate	K	AICc	ΔAICc	ModLik	AICcWt	R^2
null			1	108.64	0.00	1.00	0.52	NA
Pesticide		0.07	2	110.11	1.46	0.48	0.25	0.15
Nitrogen	-0.01		2	110.98	2.33	0.31	0.16	0.00

Global	0.00	0.07	3	112.65	4.01	0.13	0.07	0.15

H.9. *Ceriodaphnia dubia* reproductive rate in water collected from ditches, modelled on inorganic nitrogen (a linear combination of square-root transformed total ammonia and square-root transformed nitrate-nitrogen + nitrite-nitrogen) and pesticides (a linear combination of square-root transformed mean atrazine, square-root transformed mean glyphosate, and square-root transformed summed mean clothianidin, imidacloprid, and thiamethoxam) in ditch water; n=27.

Model	**Nitrogen estimate**	**Pesticide estimate**	**K**	**AICc**	**ΔAICc**	**ModLik**	**AICcWt**	**R^2**
null			2	68.99	0.00	1.00	0.54	NA
Pesticide		-0.15	3	70.64	1.65	0.44	0.24	0.03
Nitrogen	0.05		3	71.42	2.42	0.30	0.16	0.00
Global	0.04	-0.14	4	73.35	4.35	0.11	0.06	0.04

Appendix I. Trait state assignments for seven traits (body size, degree of body armouring, feeding guild, habit, oxygen acquisition, dispersal mode, and voltinism; see descriptions in Table 4.1) to 35 aquatic macroinvertebrate families collected from 27 agricultural drainage ditches in Eastern Ontario. Source number corresponds to the reference(s) used to determine trait state assignments for each family.

Order	Family	Size	Armouring	Feed guild	Habit	Oxygen	Dispersal	Voltinism	Source
Amphipoda	Crangonyctidae	large	all	other	crawl	water	passive	uni	1, 3, 11, 12, 13, 14
	Gammaridae	large	all	other	crawl	water	passive	uni	1, 3, 11, 12, 13, 14
	Hyalellidae	small	all	filter	crawl	water	passive	multi	1, 3, 11, 12, 13, 14
Basommatophora	Lymnaeidae	large	part	other	crawl	atmosphere	passive	uni	1, 3, 11
	Planorbidae	small	part	other	crawl	atmosphere	passive	multi	1, 3
Coleoptera	Dytiscidae	small	all	predator	swim	atmosphere	active	uni	1, 2
	Elmidae	small	all	other	crawl	water	active	uni	1, 3
	Haliplidae	small	all	other	crawl	atmosphere	active	multi	1, 2
	Hydrophilidae	small	all	other	crawl	atmosphere	active	uni	1, 3
Decapoda	Cambaridae	large	all	other	crawl	water	active	uni	1, 4, 5, 6
Diptera	Chironomidae	small	part	other	crawl	water	passive	multi	1, 7
	Culicidae	small	part	other	crawl	atmosphere	active	multi	1, 3
	Dixidae	small	part	filter	swim	atmosphere	passive	uni	1, 3
	Simuliidae	small	part	filter	crawl	water	active	multi	1, 8
	Tipulidae	large	part	other	burrow	water	active	uni	1, 2, 3
Ephemeroptera	Baetidae	small	part	other	swim	water	active	multi	1, 3
	Caenidae	small	part	other	burrow	water	active	multi	1, 3
	Leptophlebiidae	small	part	other	crawl	water	active	uni	1
	Siphlonuridae	large	part	other	swim	water	active	uni	1, 2

Hemiptera	Belostomatidae	large	part	predator	crawl	atmosphere	active	multi	1, 3
	Corixidae	small	part	other	swim	atmosphere	active	multi	1, 3
	Gerridae	small	part	predator	swim	atmosphere	active	uni	1, 2, 3
	Gyrinidae	small	all	other	swim	atmosphere	active	uni	1, 3
	Nepidae	large	part	predator	crawl	atmosphere	active	uni	1, 2
Heterostropha	Valvatidae	small	part	other	crawl	water	passive	multi	1, 9
Hirudinida	Erpobdellidae	large	part	predator	crawl	water	active	uni	1, 4, 5, 6
	Glossiphoniidae	large	part	predator	crawl	water	active	uni	1, 4, 5, 6
Isopoda	Asellidae	large	all	other	crawl	water	passive	uni	1, 3, 4, 5, 6
Odonata	Aeshnidae	large	all	predator	crawl	water	active	uni	1, 3
	Coenagrionidae	large	part	predator	crawl	water	active	multi	1, 3
	Lestidae	large	part	predator	crawl	water	active	uni	1, 3
	Libellulidae	large	all	predator	crawl	water	active	uni	1, 3
Trichoptera	Hydropsychidae	small	part	filter	crawl	water	active	uni	1, 2
	Limnephilidae	large	part	other	crawl	water	active	uni	1, 3
Veneroida	Sphaeriidae	small	all	filter	burrow	water	passive	uni	1, 10, 11

Source references:

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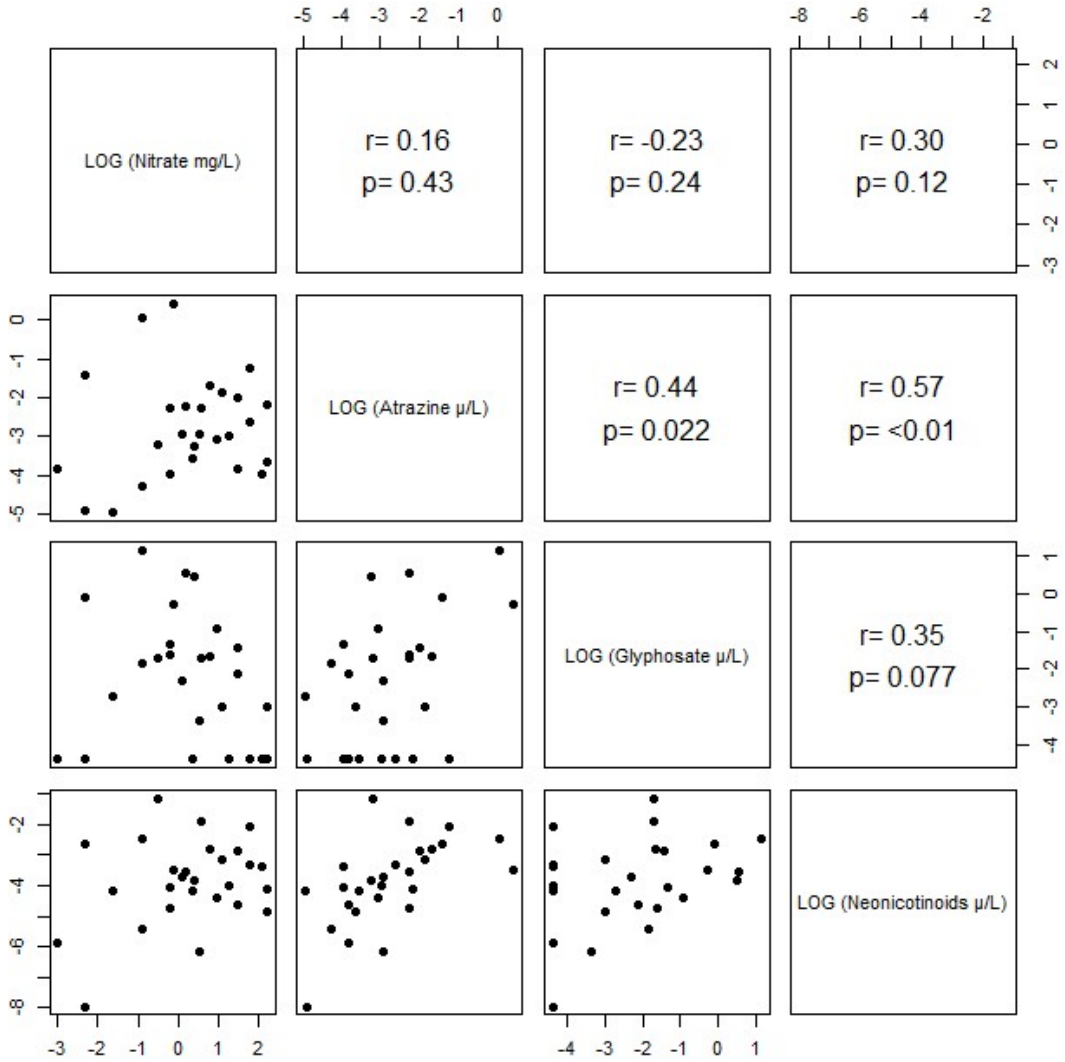
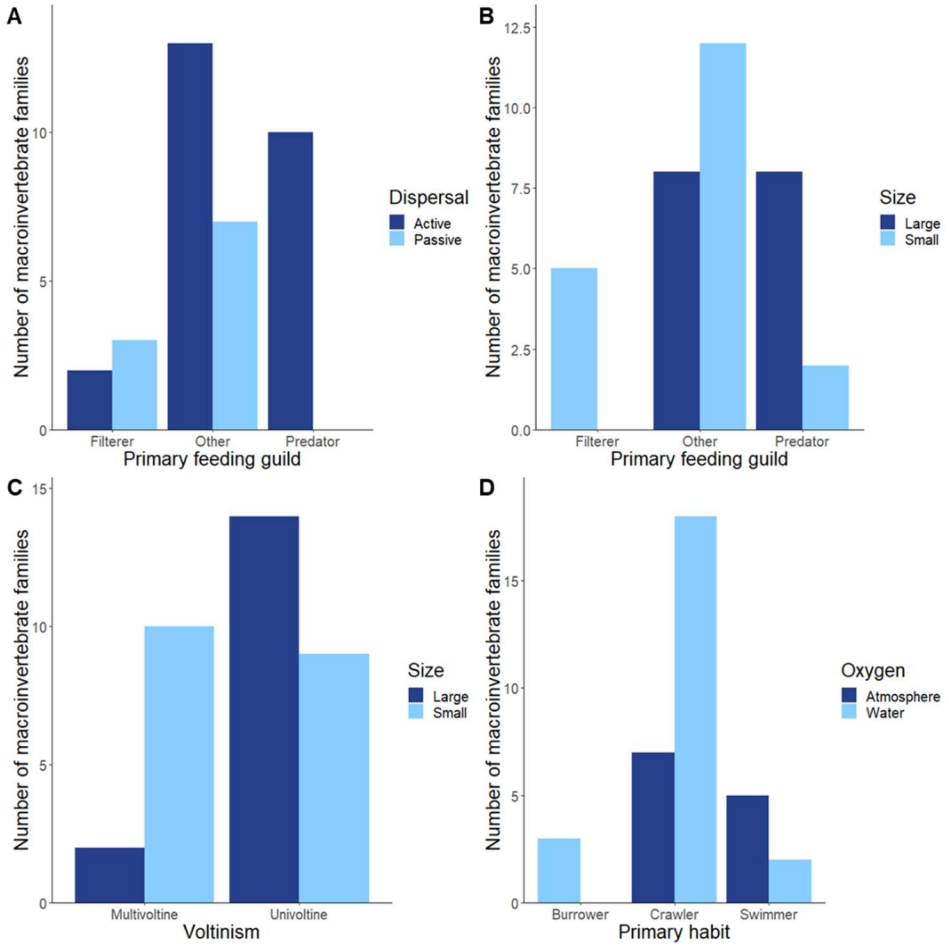
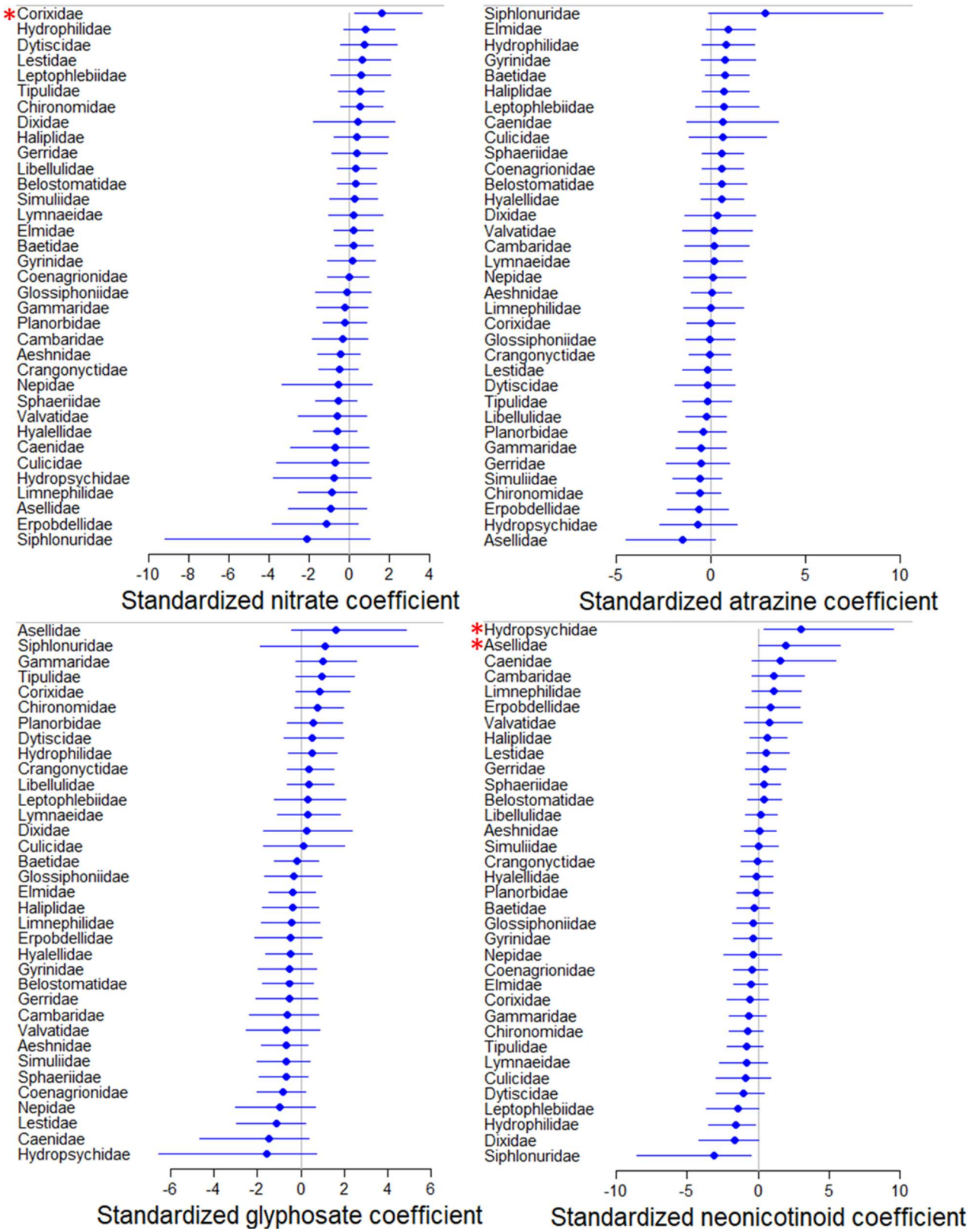
Appendix J. Pairwise correlations between four types of agrichemicals measured in drainage ditch water samples.



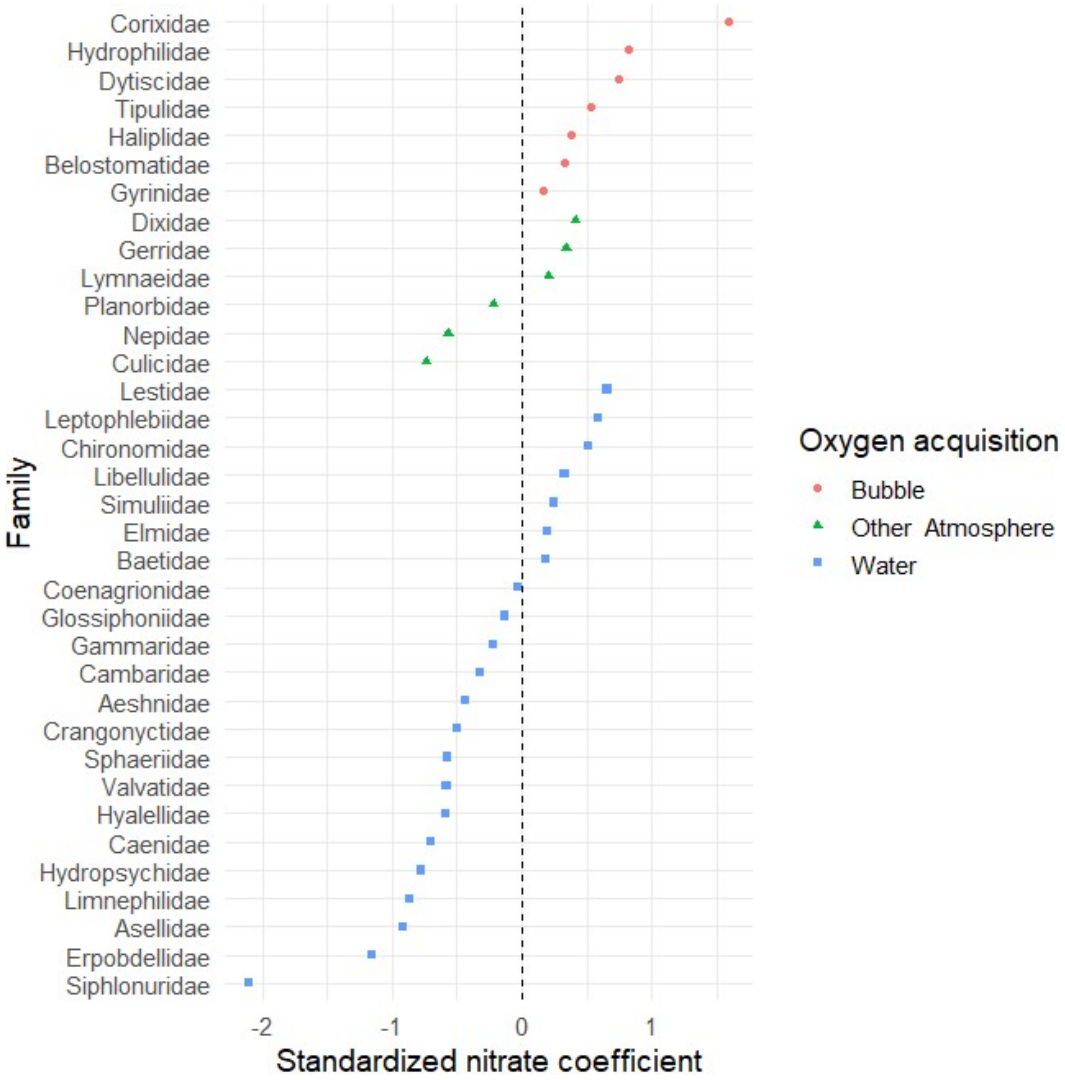
Appendix K. Significant pairwise dependencies between macroinvertebrate family traits from 35 families collected from agricultural drainage ditches. (a) A significant relationship between feeding guild and dispersal mode, driven by all predators being active dispersers. (b) A significant relationship between feeding guild and body size, driven by all filter feeders being small-bodied and most predators classified as large. (c) A significant relationship between voltinism and body size, with most small-bodied being multivoltine. (d) A strong relationship between habit and oxygen acquisition, with most crawler and burrower families acquiring oxygen from the water, while most swimmers using oxygen acquired from the atmosphere.



Appendix L. Standardized coefficients and 95% confidence intervals for each agrichemical predictor in binomial models of macroinvertebrate family absence/presence on the agrichemical concentrations in farm ditch water. Positive coefficients with non-zero-containing confidence intervals are highlighted to identify potentially useful indicator taxa of elevated levels of particular agrichemicals. Note that Corixidae was also revealed as a significant indicator of whether nitrate-nitrogen levels exceeded a proposed protected total nitrogen benchmark of 1.86 mg/L (Morgan and Kline, 2011) by indicator species analysis (De Cáceres and Legendre, 2009).


*Corixidae
Hydrophilidae
Dytiscidae
Lestidae
Leptophlebiidae
Tipulidae
Chironomidae
Dixidae
Haliplidae
Gerridae
Libellulidae
Belostomatidae
Simuliidae
Lymnaeidae
Elmidae
Baetidae
Gyrinidae
Coenagrionidae
Glossiphoniidae
Gammaridae
Planorbidae
Cambaridae
Aeshnidae
Crangonyctidae
Nepidae
Sphaeriidae
Valvatidae
Hyalellidae
Caenidae
Culicidae
Hydropsychidae
Limnephilidae
Asellidae
Erpobdellidae
Siphlonuridae
-10 -8 -6 -4 -2 0 2 4
Standardized nitrate coefficient
Siphlonuridae
Elmidae
Hydrophilidae
Gyrinidae
Baetidae
Haliplidae
Leptophlebiidae
Caenidae
Culicidae
Sphaeriidae
Coenagrionidae
Belostomatidae
Hyalellidae
Dixidae
Valvatidae
Cambaridae
Lymnaeidae
Nepidae
Aeshnidae
Limnephilidae
Corixidae
Glossiphoniidae
Crangonyctidae
Lestidae
Dytiscidae
Tipulidae
Libellulidae
Planorbidae
Gammaridae
Gerridae
Simuliidae
Chironomidae
Erpobdellidae
Hydropsychidae
Asellidae
-5 0 5 10
Standardized atrazine coefficient
Asellidae
Siphlonuridae
Gammaridae
Tipulidae
Corixidae
Chironomidae
Planorbidae
Dytiscidae
Hydrophilidae
Crangonyctidae
Libellulidae
Leptophlebiidae
Lymnaeidae
Dixidae
Culicidae
Baetidae
Glossiphoniidae
Elmidae
Haliplidae
Limnephilidae
Erpobdellidae
Hyalellidae
Gyrinidae
Belostomatidae
Gerridae
Cambaridae
Valvatidae
Aeshnidae
Simuliidae
Sphaeriidae
Coenagrionidae
Nepidae
Lestidae
Caenidae
Hydropsychidae
-6 -4 -2 0 2 4 6
Standardized glyphosate coefficient
*Hydropsychidae
*Asellidae
Caenidae
Cambaridae
Limnephilidae
Erpobdellidae
Valvatidae
Haliplidae
Lestidae
Gerridae
Sphaeriidae
Belostomatidae
Libellulidae
Aeshnidae
Simuliidae
Crangonyctidae
Hyalellidae
Planorbidae
Baetidae
Glossiphoniidae
Gyrinidae
Nepidae
Coenagrionidae
Elmidae
Corixidae
Gammaridae
Chironomidae
Tipulidae
Lymnaeidae
Culicidae
Dytiscidae
Leptophlebiidae
Hydrophilidae
Dixidae
Siphlonuridae
-10 -5 0 5 10
Standardized neonicotinoid coefficient

Appendix M. Standardized nitrate coefficients from each macroinvertebrate family binomial generalized linear model, showing families that breath via respiratory bubbles, other forms of atmospheric-breathing (e.g. siphon), and water (i.e. dissolved-oxygen breathing). All respiratory bubble-breathers have positive nitrate sensitivity coefficients, i.e. indicating a higher probability of absence with increasing nitrate levels.



Appendix N. Relative abundance data for the eight anuran species detected across 34 survey points, and four landscape variables measured within 1-km radius agricultural landscapes centered on each survey point. Relative abundance for each species was estimated as the summed chorus abundance ranks (see Chapter 2 methods) recorded at each point over four auditory surveys conducted in spring 2012. Percent forest and percent soybean are the total amounts of forest cover and soybean cover, respectively, within each of the landscapes. Crop diversity is the Shannon diversity of crop types in each landscape, and mean field size is the mean crop field size in each landscape.

Landscape	***P. cruc.***	***A. amer.***	***H. vers.***	***L. pipi.***	***L. clam.***	***L. sylv.***	***P. tris.***	***L. cate.***	**% Forest**	**% Soybean**	**Crop diversity**	**Mean field size (ha)**
2	6	8	2	1	0	4	0	0	6.75	16.91	1.50	4.16
3	5	2	0	0	0	3	0	0	22.71	44.65	0.76	5.84
7	0	0	0	0	0	0	0	0	6.35	4.11	0.82	6.53
8	2	1	0	0	0	0	0	0	10.35	27.90	1.21	5.16
9	1	0	0	0	0	0	0	0	0.87	21.03	0.81	12.86
18	1	4	0	0	0	0	0	0	3.75	27.85	1.21	7.32
19	2	3	0	0	2	0	1	0	13.72	21.93	1.36	4.87
20	1	9	0	0	0	0	0	0	8.33	24.54	0.67	8.06
22	5	1	0	0	0	0	0	0	1.43	16.28	1.48	3.08
29	1	3	0	0	0	0	0	0	0.20	25.28	0.96	8.16
31	4	2	1	0	0	0	0	0	16.16	24.96	1.30	3.25
35	3	4	1	0	1	0	1	0	29.98	12.36	0.69	4.43
36	2	6	0	0	0	0	0	0	14.79	18.80	1.55	2.28
39	4	9	4	1	1	0	0	0	15.10	12.07	1.71	3.53

40	9	5	0	2	1	0	0	0	16.12	12.37	1.50	3.39
59	3	2	0	0	1	0	0	0	10.25	28.84	1.17	6.89
72	4	1	0	3	0	0	0	0	1.35	1.27	1.32	3.15
73	3	4	2	0	1	0	0	0	1.55	35.38	1.04	11.75
74	0	6	2	1	0	0	0	0	0.00	47.46	0.95	6.64
79	4	10	3	2	0	1	1	0	18.80	15.32	1.54	2.63
80	1	2	0	0	0	0	0	0	7.63	45.54	0.71	15.89
82	1	3	0	0	0	0	0	0	3.32	17.89	1.52	5.08
87	1	2	0	0	0	0	0	0	0.00	26.50	1.55	6.62
91	9	7	2	0	0	0	0	0	11.42	11.86	1.10	3.95
92	4	6	1	0	5	0	0	3	14.05	11.60	1.67	2.17
105	4	0	0	0	0	0	0	0	3.80	22.74	0.72	4.47
118	6	5	6	0	0	0	0	0	26.90	12.16	1.09	4.76
203	5	8	5	2	0	2	0	0	17.12	21.25	1.39	4.91
205	10	6	0	3	0	3	5	0	9.45	0.00	1.35	2.04
207	0	0	2	0	0	0	0	0	0.00	32.34	1.06	4.68
211	7	5	2	0	0	0	0	0	6.68	5.85	1.71	2.06
214	0	4	2	1	0	0	0	0	2.66	12.44	1.68	4.78
215	1	1	0	0	0	0	0	0	0.00	14.74	1.52	5.11
217	7	3	0	0	0	0	0	0	5.40	18.18	1.73	4.28

Appendix O. Values of physical characteristics and general water quality parameters measured for each of the 27 sampled agricultural drainage ditch sites.

O.1. Values of physical characteristics measured during the June 2014 sampling period for each of the 27 sampled agricultural drainage ditch sites. Vegetated margin width is the average width of the uncultivated vegetation boarding one side of the ditch, from the edge of the crop field to the water, measured at three locations corresponding to the 0, 5, and 10 m points along the 10-m ditch sampling transect (see Chapter 3 methods). Channel height, channel width, and water depth are the average values from measurements recorded at three points (0, 5, and 10 m) along the sampling transect for each ditch. Percent emergent vegetation, percent submergent vegetation, and percent *Lemna* are the average values from three estimates of the percent cover of each aquatic vegetation type in 1 x 1-m quadrats centered on three points (0, 5, and 10 m) along the sampling transect for each ditch. Flow represents an estimate of the speed at which water was moving, measured by the amount of time it took for a float to travel a distance of 1 m in the water. Adjacent crop type(s) is a list of the crop types that were immediately adjacent to each side of the ditch. Vegetated margin composition is a description of the main types of uncultivated vegetation that comprised the vegetated margin boarding each side of the ditch. Town is the town/village in Eastern Ontario that the drainage ditch was located in. Note that sampling locations cannot be made public due to confidentially agreements with landowners.

Site	Average							Flow (sec/m)	Adjacent crop type(s)	Vegetated margin composition	Town
	Vegetated margin width (m)	Channel height (m)	Channel width (m)	Water depth (cm)	% Emergent vegetation	% Submergent vegetation	% Lemna				
1	NA	2.90	6.27	37.7	0	0	0	8.92	Hay	Hay field to edge	Lunenburg
2	7.22	1.52	1.64	13.3	0	0	0	6.1	Corn	Grasses & forbs	Finch
3	3.80	1.59	1.00	5.3	2	100	0	0	Corn & soy	Some trees	Crysler
4	3.87	0.93	0.97	12.7	30	15	0	0	Soy	Forbs & trees	St-Albert
5	3.30	1.07	1.88	34.7	5	70	0	0	Clover & soy	Grasses & forbs	Iroquois
6	3.65	1.15	1.76	16.0	80	50	0	0	Corn & hay	Grasses	Brinston
7	4.57	1.08	1.85	23.7	10	42	0	60	Soy	Grasses	Iroquois
8	4.30	1.59	1.76	22.7	58	12	0	0	Corn, hay, & soy	Grasses	Williamsburg
9	8.67	1.88	1.30	22.0	27	7	0	0	Corn & soy	Grasses	Winchester
10	11.57	0.98	1.36	14.7	8	63	10	0	Soy	Grasses	Winchester
11	6.93	2.33	1.70	17.7	32	70	0	4.81	Soy, hay, & corn	Grasses & forbs	Inkerman
12	4.57	1.85	2.64	36.0	8	12	0	14.9	Soy & corn	Forbs	Brinston
13	NA	1.25	0.95	19.0	5	90	0	0	Pasture	Hay field to edge, treed other side	Oxford Mills
14	7.93	1.18	1.34	19.3	8	42	0	0	Corn	Grasses & forbs	North Gower
15	3.13	1.16	2.10	20.3	25	18	0	0	Soy	Grasses & some trees	Winchester
16	2.83	1.63	1.30	18.7	32	100	0	0	Clover & corn	Grasses	Metcalfe
17	5.70	1.44	1.56	5.0	3	3	0	0	Soy & hay	Forbs & trees	Osgoode
18	7.50	2.15	2.85	27.0	0	22	0	3.18	Clover, corn, soy, hay	Grasses, forbs, some trees	Crysler
19	6.80	0.94	1.59	17.7	63	10	8	0	Apples, corn, vegetables	Treed & short mowed grass	Crysler
20	6.65	2.23	1.42	17.3	17	12	1	0	Soy	Grasses & forbs	Chesterville
21	7.20	2.30	3.25	29.7	72	2	0	10	Corn, soy & hay/grass	Grasses	Winchester
22	4.47	2.00	2.55	14.3	5	18	1	11.08	Soy	Grasses & forbs	Russell
23	3.61	3.13	2.04	27.3	7	5	0	14.04	Soy, corn & hay	Grasses & some trees	Dunvegan

24	3.46	1.40	0.88	13.0	57	2	0	0	Corn	Grasses & forbs	North Glengarry
25	3.98	2.32	0.99	18.7	15	5	0	13.46	Soy & corn	Grasses	Saint Isidore
26	5.67	1.80	1.62	35.0	8	20	4	0	Corn & tree lot	Forbs & trees	Iroquois
28	3.43	2.02	2.72	53.7	38	67	47	0	Corn & grass/hay	Grasses & forbs	Richmond

O.2. Values of general water quality parameters measured during the June and July 2014 sampling periods for each of the 27 sampled agricultural drainage ditch sites. Dissolved oxygen (DO) was measured using a Horiba Scientific LAQUAact DO Meter OM-71, and pH and water temperature were measured using a Horiba Scientific LAQUAact pH meter. Note that site 27 was dry in July and therefore excluded from analyses.

Site	Date	DO (mg/L)	pH	Water temp (°C)
1	JN 6	8.62	7.2	16.3
2	JN 6	9.87	7.2	13.5
3	JN 6	4.3	6.5	14.5
4	JN 6	7	7.1	18.2
5	JN 7	14.58	7.2	16.5
6	JN 7	2.45	7.2	19.5
7	JN 7	6.39	7.1	16.7
8	JN 7	13.62	7.1	23.3
9	JN 7	6.53	7.1	17.5
10	JN 8	2.96	7.2	16.3
11	JN 8	17.19	7.2	19.3
12	JN 8	15.11	7.5	23.2
13	JN 8	3.2	7.1	21.8
14	JN 8	11.54	7.1	23.2
15	JN 9	5.01	7.2	16.6
16	JN 9	11.97	7.1	22.5
17	JN 9	3	7.6	21.9
18	JN 10	6.64	7.2	19.6
19	JN 10	7.55	7	14.1
20	JN 10	13.29	7.3	21.5
21	JN 10	17.27	7.2	24.6
22	JN 10	11.5	7.3	24.8
23	JN 11	13.27	7.7	14.9
24	JN 11	1.49	8.2	16.8
25	JN 11	9.64	7.7	19
26	JN 12	7.21	7.2	15.2
27	JN 12	1.69	7.3	17.7
28	JN 12	6.94	N/A	13.2
1	JL 7	7.15	7.6	21.4
2	JL 7	7.57	7.5	18
3	JL 7	4.46	6.7	15.3
4	JL 7	7.11	7.3	18.5

5	JL 8	1.89	6.7	21.8
6	JL 8	2.76	6.7	18
7	JL 8	11.87	6.6	20.4
8	JL 8	14.1	6.3	22.1
9	JL 8	5.32	6.4	15.7
10	JL 9	1.64	6.7	17.9
11	JL 9	10.39	6.7	19.3
12	JL 9	7.52	6.7	20.2
13	JL 9	15.14	6.5	18.5
14	JL 9	10.79	6.5	20.3
15	JL 10	10.3	6.8	16.3
16	JL 10	20	6.8	21.8
17	JL 10	7.43	6.8	18.5
18	JL 11	9.04	7.3	17.5
19	JL 11	10.51	6.9	16.9
20	JL 11	9.67	7.1	18.6
21	JL 11	15.56	7	22
22	JL 11	9.78	6.8	19.8
23	JL 12	13.84	7.5	16.7
24	JL 12	2.13	7.3	17.3
25	JL 12	10.47	7.3	19.7
26	JL 10	0.23	6.9	16.9
28	JL 12	14.3	7.2	17.4

Appendix P. Concentrations of agrichemicals measured in water samples collected from agricultural drainage ditches in June and July 2014.

P.1. Concentrations of atrazine, glyphosate, clothianidin, imidacloprid, and thiamethoxam measured in water samples collected from 28 agricultural drainage ditch sites in June and July 2014. Concentrations were determined using high-performance liquid chromatography and tandem mass spectrometry methods at the National Wildlife Research Centre (NWRC) in Ottawa, Ontario, Canada. "LOD" = limit of detection. Refer to Chapter 3 methods for more information. Note that site 27 was dry in July and thus excluded from analyses.

Site	Date collected	Atrazine (ppb)	Glyphosate (ppb)	Clothianidin (ppb)	Imidacloprid (ppb)	Thiamethoxam (ppb)
1	JN 6	1.8E-02	2.7E-01	1.9E-03	< LOD	1.3E-03
2	JN 6	5.4E-02	< LOD	7.4E-04	< LOD	7.0E-04
3	JN 6	1.0E-01	< LOD	1.5E-02	< LOD	1.3E-02
4	JN 6	2.8E+00	5.6E-02	1.2E-02	< LOD	8.5E-03
5	JN 7	1.9E-02	< LOD	1.8E-03	< LOD	< LOD
6	JN 7	2.4E-02	< LOD	6.4E-03	< LOD	6.9E-04
7	JN 7	9.1E-03	< LOD	9.0E-03	< LOD	1.6E-03
8	JN 7	2.0E-02	5.2E-02	3.1E-03	3.7E-04	1.5E-03
9	JN 7	7.7E-02	6.3E-01	8.5E-03	< LOD	4.4E-03
10	JN 8	1.2E-02	3.2E-02	4.8E-03	< LOD	7.2E-03
11	JN 8	9.7E-02	3.8E-02	1.2E-02	< LOD	2.2E-03
12	JN 8	2.3E-01	1.3E-01	2.4E-02	< LOD	9.4E-03
13	JN 8	5.0E-03	< LOD	< LOD	< LOD	< LOD
14	JN 8	9.0E-03	< LOD	1.9E-02	< LOD	4.6E-03
15	JN 9	5.7E-02	< LOD	5.6E-03	< LOD	4.4E-03
16	JN 9	5.3E-02	< LOD	8.1E-03	< LOD	1.0E-03
17	JN 9	1.8E-02	< LOD	1.2E-02	1.0E-03	8.0E-03
18	JN 10	9.8E-02	6.6E-02	6.8E-04	4.5E-04	6.5E-04
19	JN 10	1.3E-01	< LOD	9.2E-03	< LOD	1.4E-03
20	JN 10	1.4E-01	1.9E-01	8.6E-03	2.0E-03	4.6E-03
21	JN 10	5.0E-02	6.3E-02	6.7E-03	4.3E-04	2.3E-03
22	JN 10	2.2E-02	5.1E-01	3.6E-03	< LOD	2.0E-03
23	JN 11	1.6E-02	< LOD	5.3E-03	< LOD	4.0E-03

24	JN 11	4.5E-02	1.8E+00	1.9E-02	< LOD	1.2E-02
25	JN 11	1.7E+00	1.0E-01	1.5E-02	< LOD	9.7E-03
26	JN 12	6.5E-02	2.3E-01	4.2E-01	< LOD	1.9E-01
27	JN 12	2.1E-01	1.3E-01	8.8E-03	1.0E-03	1.1E-02
28	JN 12	4.7E-01	< LOD	1.1E-01	< LOD	7.5E-02
1	JL 7	1.0E-02	4.9E-02	2.1E-03	< LOD	3.4E-03
2	JL 7	5.4E-02	5.6E-02	1.7E-03	3.1E-04	6.8E-04
3	JL 7	1.7E-01	4.7E-01	3.1E-02	< LOD	5.6E-02
4	JL 7	2.9E-01	1.4E+00	1.8E-02	< LOD	2.1E-02
5	JL 8	2.6E-02	< LOD	2.5E-03	< LOD	1.0E-03
6	JL 8	2.9E-02	8.7E-02	7.2E-03	< LOD	1.4E-03
7	JL 8	5.4E-03	1.2E-01	9.4E-03	< LOD	9.7E-03
8	JL 8	2.4E-02	1.9E-01	5.2E-03	6.9E-04	8.7E-03
9	JL 8	1.8E-02	1.7E-01	8.8E-03	< LOD	2.9E-03
10	JL 9	6.6E-02	3.2E+00	9.6E-03	4.6E-03	1.6E-02
11	JL 9	1.1E-01	3.3E-01	4.5E-02	6.2E-03	2.3E-01
12	JL 9	1.5E-01	2.4E-01	3.1E-02	3.8E-04	5.8E-02
13	JL 9	9.8E-03	< LOD	< LOD	< LOD	< LOD
14	JL 9	3.0E-01	8.7E-02	3.0E-02	< LOD	3.4E-02
15	JL 10	8.8E-02	< LOD	1.5E-02	8.3E-04	4.6E-02
16	JL 10	5.0E-02	< LOD	1.7E-02	3.5E-04	9.2E-03
17	JL 10	2.0E-02	< LOD	2.2E-02	6.4E-03	1.9E-02
18	JL 11	1.1E-01	3.3E-01	6.1E-03	< LOD	9.9E-03
19	JL 11	1.0E-01	< LOD	8.7E-03	1.2E-02	9.6E-04
20	JL 11	6.8E-02	3.2E+00	1.9E-02	6.4E-03	1.8E-02
21	JL 11	5.7E-02	1.4E-01	1.5E-02	1.2E-03	2.3E-02
22	JL 11	1.6E-02	< LOD	7.6E-03	1.1E-03	2.0E-02
23	JL 12	4.1E-02	< LOD	8.9E-03	5.8E-04	1.2E-02
24	JL 12	4.5E-01	< LOD	2.8E-02	3.4E-04	8.4E-02
25	JL 12	3.5E-01	6.2E+00	9.1E-02	2.1E-03	4.7E-02
26	JL 10	1.6E-02	1.4E-01	2.0E-02	3.0E-04	4.9E-03
28	JL 12	1.1E-01	< LOD	3.7E-02	< LOD	3.0E-02

P.2. Concentrations of nitrite-nitrogen (NO_2–N), nitrate-nitrogen (NO_3–N), and total ammonia nitrogen (TAN: NH_3–N and NH_4–N) measured in water samples collected from 27 agricultural drainage ditch sites in July 2014. Concentrations were determined following standard methods of the Environment Laboratory Services Branch of the Ontario Ministry of the Environment at Caduceon Environmental Laboratories in Ottawa, Ontario, Canada. "LOD" = limit of detection. Refer to Chapter 3 methods for more information.

Site	Date collected	NO_2-N (mg/L)	NO_3-N (mg/L)	TAN (mg/L)
1	JL 7	< LOD	0.4	0.03
2	JL 7	< LOD	1.7	0.01
3	JL 7	< LOD	4.3	0.37
4	JL 7	< LOD	0.9	0.02
5	JL 8	< LOD	< LOD	< LOD
6	JL 8	< LOD	9	0.13
7	JL 8	< LOD	0.2	0.01
8	JL 8	< LOD	4.3	0.02
9	JL 8	< LOD	2.6	0.13
10	JL 9	0.3	1.5	0.01
11	JL 9	< LOD	1.8	0.03
12	JL 9	0.2	2.2	0.03
13	JL 9	< LOD	0.1	0.03
14	JL 9	< LOD	3	0.03
15	JL 10	< LOD	6	0.02
16	JL 10	< LOD	3.5	< LOD
17	JL 10	< LOD	8	< LOD
18	JL 11	< LOD	0.8	< LOD
19	JL 11	< LOD	9	< LOD
20	JL 11	< LOD	1.2	< LOD
21	JL 11	< LOD	1.1	< LOD
22	JL 11	< LOD	0.8	< LOD
23	JL 12	< LOD	1.4	< LOD
24	JL 12	< LOD	0.1	0.05
25	JL 12	< LOD	0.4	< LOD
26	JL 10	< LOD	0.6	0.01
28	JL 12	< LOD	5.9	0.02

Appendix Q. Biological water quality indicators measured from 27 agricultural drainage ditch sites and surrounding landscape variables measured in 1-km radius landscapes surrounding ditch sampling sites. Daphnia survival and reproduction were population responses of *Ceriodaphnia dubia* to ditch water exposure in 27 laboratory bioassays using water collected from each ditch during the June 2014 sampling period. Daphnia survival is the total number of test individuals (of 10) alive at the end of each bioassay and daphnia reproduction is the average number of offspring produced per live individual ($n \leq 10$), per day, calculated for each 8-day bioassay. Leaf litter weight loss is the amount of weight lost from 3.00 g of leaf litter enclosed in a mesh bag and exposed to water in the ditch for one month as an assessment of litter decomposition rate in each ditch. Note that bags from sites 11 and 15 were not retrieved. Invertebrate richness is the total number of aquatic macroinvertebrate families per site sampled from both collection periods in 2014. Percent forest is the total amount of forest cover within each landscape and percent field edge cover is the total amount of crop field edge cover within each landscape. Percent high-intensity crop cover is the total amount of the major crop types associated with high agrichemical inputs—corn, soy, and cereals—in each landscape. See Chapter 3 methods for more details.

Site	Daphnia survival	Daphnia reproduction	Leaf litter weight loss (g)	Invertebrate richness	% Forest	% Field edge	% High-intensity crop
1	7	3.34	1.04	22	21.78	5.87	5.04
2	5	2.64	1.58	15	24.26	2.83	45.07
3	7	2.62	1.13	7	0.00	2.27	86.23
4	8	3.50	2.24	12	0.70	4.21	53.83
5	7	2.51	0.78	13	16.57	4.72	38.19
6	6	2.47	1.60	8	10.86	3.25	73.39

7	5	1.95	1.58	16	15.13	3.55	54.35
8	6	2.80	1.55	20	9.49	2.53	82.72
9	6	2.70	1.81	9	2.53	5.13	49.15
10	5	4.55	0.79	15	6.59	4.93	52.51
11	6	2.76	NA	19	0.00	6.50	72.90
12	7	2.56	2.15	21	2.94	5.01	72.33
13	5	3.60	1.04	13	7.47	6.03	16.65
14	5	3.04	0.84	16	10.41	3.25	60.64
15	9	4.83	NA	15	3.64	3.18	45.89
16	7	5.00	2.53	10	23.39	3.55	42.11
17	5	4.40	2.78	15	12.64	4.43	50.17
18	8	3.12	2.52	22	9.90	6.31	40.19
19	6	3.31	1.50	15	11.31	6.27	47.47
20	7	2.81	2.42	20	7.64	3.22	81.28
21	8	4.27	0.79	22	2.70	2.17	72.99
22	6	2.66	0.96	20	3.43	3.42	61.63
23	6	2.75	1.31	16	0.00	3.74	67.46
24	6	2.67	1.02	13	5.28	3.73	50.82
25	8	2.23	1.44	13	0.00	3.70	51.98
26	7	2.67	1.45	13	34.19	2.49	37.76
28	9	3.72	0.56	14	0.00	3.51	81.84

Appendix R. Numbers of individuals of each aquatic macroinvertebrate taxa sampled from all 27 agricultural drainage ditch sites, across two collection periods in June and July 2014. Note that numbers of non-insects cannot be used as absolute values due to rapid assessment subsampling protocols applied to highly abundant taxa during sampling.

			Site																										
Class	**Order**	**Family**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**28**
Bivalvia	Veneroida	Sphaeriidae	1	4	5	0	0	4	3	0	1	6	0	2	0	0	2	0	0	7	6	0	4	5	2	5	0	0	0
Clitellata	Arhynchobdellida	Erpobdellidae	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2	2	0	0	0	0	0	0	0	0
	Rhynchobdellida	Glossiphoniidae	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	2	0	0	1	0	0	0	0
Gastropoda	Basommatophora	Bithyniidae	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Hydrobiidae	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Lymnaeidae	0	0	2	1	5	1	6	9	6	8	6	0	10	6	5	3	2	3	6	6	0	4	1	5	4	2	14
		Physidae	4	4	6	7	6	7	1	5	6	2	5	2	1	4	7	5	2	7	0	0	7	5	6	7	1	4	7
		Planorbidae	0	4	0	2	3	0	2	3	1	6	1	3	0	1	1	2	2	4	1	7	7	7	2	6	0	0	4
		Valvatidae	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	4	0	0	3	0	0	0	0	0	0
		Viviparidae	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Curculionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		Dytiscidae	0	0	0	14	6	7	21	1	0	12	8	4	7	8	8	3	5	2	11	5	8	17	0	6	11	3	14
		Elmidae	2	2	0	0	0	0	2	2	0	0	2	2	0	0	0	0	0	4	0	0	2	0	0	0	0	1	0
		Gyrinidae	0	0	0	0	0	0	2	0	1	0	1	0	0	0	0	0	0	4	0	0	5	0	0	0	0	0	0
		Haliplidae	1	1	0	0	4	0	7	4	1	7	5	7	14	7	2	6	0	0	0	9	8	2	1	0	12	0	0
		Hydrophilidae	0	0	0	0	9	1	3	2	0	12	9	4	3	3	5	1	3	0	0	2	16	0	0	15	2	3	5
		Psephenidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Diptera	Chironomidae	1	8	0	1	0	0	4	0	0	0	0	0	0	2	1	0	1	0	15	1	7	0	1	3	2	1	1
		Culicidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
		Dixidae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0

	Empididae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Pelecorhynchidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Simuliidae	0	0	0	1	0	0	0	0	0	0	4	0	0	0	0	0	0	11	0	4	1	5	1	0	1	0	0
	Stratiomyidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0
	Tabanidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Tipulidae	0	0	0	1	1	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	1
Ephemeroptera	Ameletidae	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	Baetidae	5	4	0	0	5	0	1	1	0	2	1	13	0	1	0	0	0	0	0	33	3	3	9	0	0	0	2
	Caenidae	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Ephemerellidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	Heptageniidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leptophlebiidae	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	1	0
	Siphlonuridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	1	0
Hemiptera	Belostomatidae	0	0	0	0	8	0	0	2	0	3	5	13	2	0	0	0	0	1	0	0	10	1	0	0	0	0	0
	Corixidae	5	0	0	2	3	0	20	5	0	0	8	0	2	3	5	7	0	8	0	6	7	11	2	0	2	2	4
	Gerridae	0	1	2	1	2	0	1	2	3	5	0	1	7	5	1	1	6	1	1	5	1	4	0	6	3	4	0
	Mesoveliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	Nepidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	Notonectidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Odonata	Aeshnidae	1	4	0	0	0	0	0	1	0	0	1	14	0	1	3	0	0	0	0	2	1	6	0	0	1	0	0
	Calopterygidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	Coenagrionidae	3	0	0	0	0	0	0	1	0	6	7	1	0	0	0	0	0	4	0	15	22	2	6	3	0	0	18
	Lestidae	0	0	0	0	0	0	0	0	0	4	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	0	0
	Libellulidae	0	0	0	0	4	0	0	0	0	1	0	2	19	1	0	1	5	0	1	1	0	0	0	12	0	0	0
Plecoptera	Perlidae	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0
	Perlodidae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Trichoptera	Apataniidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Hydropsychidae	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0

		Limnephilidae	2	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0
Malacostraca	Amphipoda	Crangonyctidae	0	0	9	2	0	6	3	6	8	0	0	0	3	3	3	0	2	2	3	0	0	4	1	0	3	3	2
		Gammaridae	0	0	0	0	0	0	0	0	0	0	3	1	0	0	3	0	0	8	2	0	0	3	2	0	0	0	1
		Hyalellidae	3	0	0	0	0	1	0	2	0	0	10	15	0	0	0	0	0	3	0	10	5	3	2	0	0	0	1
	Decapoda	Cambaridae	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0
	Isopoda	Asellidae	1	7	10	9	1	7	3	4	7	0	8	2	4	13	17	10	7	5	5	1	0	8	2	0	2	0	8

Appendix S. The number of neonates produced per *Ceriodaphnia dubia* test individual per day (starting on day 3) for each 8-day bioassay. Bioassays were conducted using water collected from each of 28 drainage ditch sites in June 2014. Each bioassay consisted of recording *C. dubia* survival and reproduction in 10 replicates of 15 ml undiluted ditch water using a single *C. dubia* female in each replicate. Mortalities of test individuals are indicated in the data tables by greyed-out cells, beginning on the day mortality was recorded for a given test individual.

S.1. The number of neonates produced per day in each treatment replicate of ditch water from site 1.

	Site 1 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	0	0	5	3	1	0	3	2	0	0
5	5	4	0	0	0	5	0	4	0	7
6	2	0	12	11		3	8	4	8	5
7	0	2	12	8		2	0	4	3	1
8	3	7	0	0		6	10	9	0	10

S.2. The number of neonates produced per day in each treatment replicate of ditch water from site 2.

	Site 2 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0		0	0	0		0
4	0	0	0	0		0	0	0		0
5	0	3	2	4		0	0	3		3
6	2			0		2	8	1		0
7	0			6		1	0	7		10
8	12			15		6	3	0		0

S.3. The number of neonates produced per day in each treatment replicate of ditch water from site 3.

	Site 3 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	1	0
4		0	4	0	0		0	0	0	2
5		0	0	0	2		2	0	3	0
6		8	8		0		0	9	0	5
7		0	0		4		9	0	4	0
8		6	12		5		10	11	1	5

S.4. The number of neonates produced per day in each treatment replicate of ditch water from site 4.

	Site 4 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	0	0	4	0	4	3	0	0	0	0
5	0	0	0	0	0	1	3	0	3	4
6	13	0	1	13	10	11	11	9	8	8
7	0	4	3	8	17	0	0	0	0	1
8	8	1	0	1	0	11	13	10	13	0

S.5. The number of neonates produced per day in each treatment replicate of ditch water from site 5.

	Site 5 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	5	7	4	0	3	3	3	3	4	4
5	0	0	1	3	0	0	0	1	0	0
6	4		5	0	11	10	0	5	1	8
7	1		0	0	0	0		0	0	0
8	4		10	4	5	0		4	0	10

S.6. The number of neonates produced per day in each treatment replicate of ditch water from site 6.

	Site 6 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3		0	0	0	0	0	0	0	0	0
4		2		0	4	0	0	2		0
5		0		1	1		0	0		2
6		8		0	8		6	5		4
7		0		7	0		0	0		0
8		7		0	13		3	9		7

S.7. The number of neonates produced per day in each treatment replicate of ditch water from site 7.

	Site 7 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0		0	0	0
5	0	0	1	0	2	0		2	0	1
6	0	2	7	7	0	4		5		6
7	3	0	0	0	6	0		0		0
8	0	6	10	5	0			0		8

S.8. The number of neonates produced per day in each treatment replicate of ditch water from site 8.

	Site 8 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	3		5	0	6	0	0	0	0	3
5	0		0	2	1		2	0	2	0
6	8		0	0	5		8	7	6	9
7	0		0	8	1		0	0	0	0
8	9		12	0	0		10		0	12

S.9. The number of neonates produced per day in each treatment replicate of ditch water from site 9.

	Site 9 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0		0	
4	4	3	1	2	0	2	0		0	
5	0	0	0	0	0	0	0		0	
6	9	6	6	2	0	5	0		7	
7	13	16	3	6	0	8	5		13	
8	1	0		0	0	0	6		0	

S.10. The number of neonates produced per day in each treatment replicate of ditch water from site 10.

	Site 10 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0		0	0	0		0	0	0	0
4	5		0	5	5		1	5	6	7
5	3		0	7	7		0	0	0	0
6	0			1	1		0	1	9	8
7	0			13	13		0	8	10	11
8				11	16		0	0	15	1

S.11. The number of neonates produced per day in each treatment replicate of ditch water from site 11.

	Site 11 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3		0			0	0	0	0	0	0
4		0			0	0	4	0	1	6
5		2			1	3	0	0	0	0
6		0			0	0	3	0	4	9
7		6			0	9	9	10	9	0
8		12			8	8	0	0	0	

S.12. The number of neonates produced per day in each treatment replicate of ditch water from site 12.

	Site 12 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	1	0	0	0
4	0	0	0	0	0	0		0	0	0
5	3	5	0	1	1	0		1	1	1
6	0	0	6	0	0	5		0	0	0
7	9	2	0	8	3	1		9	7	0
8	11	9	7	5	5			5	7	

S.13. The number of neonates produced per day in each treatment replicate of ditch water from site 13.

	Site 13 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0		0	0	0			0	0	0
4	4		0	6	0			4	0	4
5	0		0	0	2			0	3	0
6	11		0	6	1			3	0	5
7	11		8	3				10	4	8
8	1		11	14				0		0

S.14. The number of neonates produced per day in each treatment replicate of ditch water from site 14.

	Site 14 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	1	0	0	0	0	0	0		0	
4	0	3	2	6	0	4	6		0	
5	0	0	0	0	0	0	0		0	
6	0	4	8	8	0	7	7		5	
7	1	16	7	1	3	0	0		1	
8		8	0		4		8		4	

S.15. The number of neonates produced per day in each treatment replicate of ditch water from site 15.

	Site 15 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	1	0	2	3	4	5	3	0	3	4
5	9	6	12	10	10	9	12	5	0	10
6	0	6	0	0	0	0	0	13	0	0
7	15	1	11	18	3	14	11	15	11	17
8	8	9	2	0	0	8	0	0	13	2

S.16. The number of neonates produced per day in each treatment replicate of ditch water from site 16.

	Site 16 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0		0		0	0	0	0
4	5	4	5		4			6	3	3
5	9	9	5		8			7	10	12
6	0	0	0		0			0	1	0
7	13	10	13		11			10	13	14
8	0	0	16		9			7	3	0

S.17. The number of neonates produced per day in each treatment replicate of ditch water from site 17.

	Site 17 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0			0	0	0	0	0	0
4	5	3			3	6	6	0	4	5
5	6	8			9	10	8		10	11
6	1	0			0		0		1	1
7	11	8			13		12		8	1
8	0	0			0		2			0

S.18. The number of neonates produced per day in each treatment replicate of ditch water from site 18.

	Site 18 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	2	0	1	0	0	0	0	0	0	
4	0	2	3	0	0	4	8	0	6	
5	6	1	0	0	0	0	0	0	0	
6	0	7	2	6	1	7	9	2	2	
7	11	0	0	13	3	0	0	4	0	
8	18	12	8	1	1	13	2	0	6	

S.19. The number of neonates produced per day in each treatment replicate of ditch water from site 19.

	Site 19 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4		0	4	2	0	0	0	5	0	6
5		1	0	0	2	2	3	0	7	0
6		1		6	4	8	3	9	10	11
7		0		10	5	0	0	12	1	9
8				0	1	8	8	0	9	0

S.20. The number of neonates produced per day in each treatment replicate of ditch water from site 20.

	Site 20 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	3	1	0	0	0	0	0	0	1	0
5	0	7	1	0	6		0	0	1	7
6	16	0	8		0		0	12	7	2
7	5	8	4		6			8	5	0
8	0	12	0		0			1	0	1

S.21. The number of neonates produced per day in each treatment replicate of ditch water from site 21.

	Site 21 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	3	0	4	5	4	0	0	5	2	0
5	0	4	8	4	10	5		11	2	6
6	13	8	0	0	0	7		0	7	0
7	16	10	15	8	8	9		2	3	
8	0	1	11	0	11	0		11	0	

S.22. The number of neonates produced per day in each treatment replicate of ditch water from site 22.

	Site 22 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0		0	0	0	0	0
4	3	4	5			7	0	0	0	0
5	0	0	0			0	3	6	3	0
6		6	6			9	3	4	3	3
7		6	9			5	1	2	0	1
8		0	3				4	0	6	5

S.23. The number of neonates produced per day in each treatment replicate of ditch water from site 23.

	Site 23 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0		0	0	0	0	0	0	0	0
4	0		0	0	0	0	2	0	0	0
5	4		1	4	5	3	0	4	4	5
6	5		6	7	13	6	7	2	3	7
7	9		8	6	8	0	1	5	2	7
8	0		0	1	0			0	0	0

S.24. The number of neonates produced per day in each treatment replicate of ditch water from site 24.

	Site 24 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	3	4	3		3			4	5	5
6	0	1	6		8			4	3	6
7		1	0		9			1	10	15
8		1	7		0			0	1	0

S.25. The number of neonates produced per day in each treatment replicate of ditch water from site 25.

	Site 25 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	4	2	0	0	2
5	8	0	0	2	6	1	0	0	1	0
6	0	0	0	3	4	2	6	4	5	1
7		9	1	8	0	0	9	9	13	4
8		2	0	2	5		1	1	0	0

S.26. The number of neonates produced per day in each treatment replicate of ditch water from site 26.

	Site 26 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	3	0	0	0	0
5	7	3	3	5	6	0	5	2	4	4
6	0	0	3	3	4	10	10		4	0
7	0	0	0	7	0	8	9		0	
8	1	3	7	0	6	1			8	

S.27. The number of neonates produced per day in each treatment replicate of ditch water from site 27.

	Site 27 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0		0	0	0	0		0	0	0
6	2		6		3	5		1	0	3
7	3		0		0	1		1	0	0
8	5		5		6	1		4	3	

S.28. The number of neonates produced per day in each treatment replicate of ditch water from site 28.

	Site 28 Treatment Replicates									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	0	0	0	0	0	0	0	0	0	0
4	6	7	3	0	0	2	3	4	4	0
5	3	11	0	5	0	0	0	8	2	6
6	2	0	7	2		12	10	0	9	1
7	13	15	3	6		13	10	11	12	10
8	0	0	0	2		0	0	0	1	1