

## Original Articles

## Are macroinvertebrate traits reliable indicators of specific agrichemicals?

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## ARTICLE INFO

## ABSTRACT

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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 warrant thorough chemical testing. Many general 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 indicators. Here, we investigate whether macroinvertebrate family-level traits can be used as simple indicators of elevated levels of specific pollutants in farmland drainage ditches, to reveal areas in need of further chemical monitoring. We selected seven traits—body size, body armouring, feeding guild, habit, oxygen acquisition, dispersal mode, and voltinism—that we predicted would influence 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 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 on the concentrations of the four agrichemicals. We reduced the seven traits predicted to influence sensitivity to four—body armouring, oxygen acquisition, dispersal mode, and voltinism—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 across families 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. In particular, the presence of Corixidae appears to indicate low ditch nitrate levels. Overall, however, our results combined with previous work suggest that we are unlikely to find chemical-specific macroinvertebrate indicators that are more efficient than a rigorous chemical sampling scheme.

## 1. 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 frequently 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 chemical concentrations. 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

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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 hot-spots” 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. to assess 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, 2011; Culp 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, 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 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). 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 certain macroinvertebrate family-level traits (Table 1) 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 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.

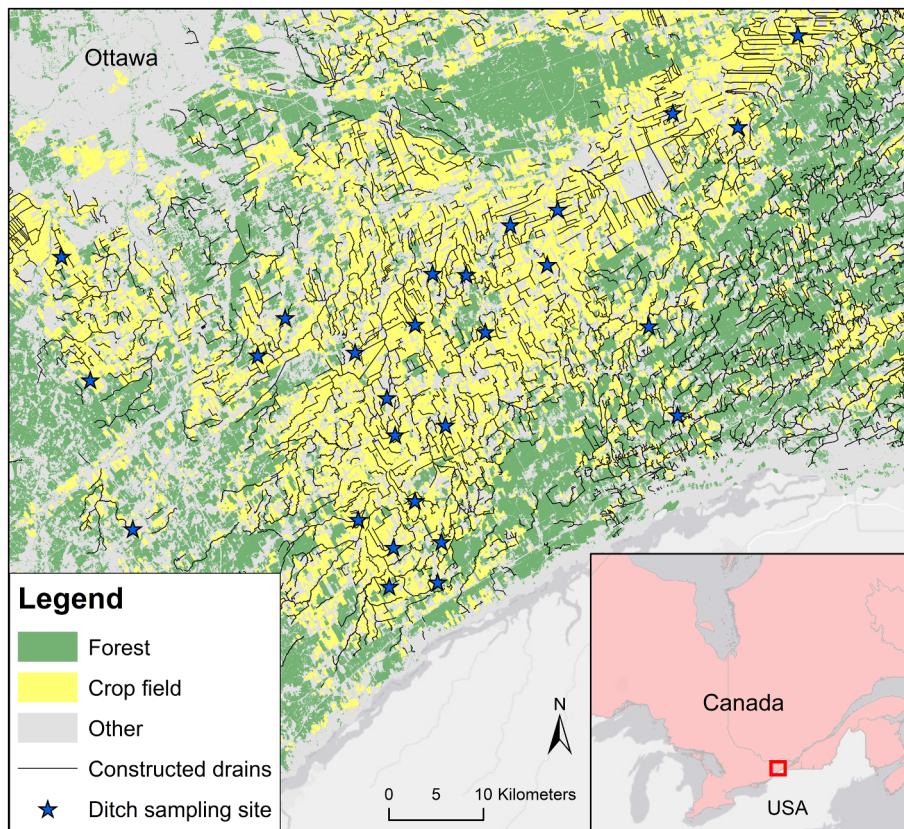
## 2. Materials and methods

### 2.1. 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. 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

**Table 1**  
Traits of aquatic invertebrates predicted to influence their sensitivity to agrochemicals.

Trait	States	Hypothesis	Prediction	Supporting literature
Body size	<i>Small</i> = < 9 mm <i>Large</i> = > 9 mm	Chemical exposure and uptake potential is higher for smaller organisms than larger ones, due to smaller organisms having higher surface area to volume ratios.	<i>Small</i> will be associated with higher sensitivity than <i>Large</i>	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	<i>Part</i> = body is only partly armoured, or soft-bodied <i>All</i> = body completely sclerotized or hard-shelled	Fully armoured organisms have lower chemical uptake and thus exposure risks than partly-armoured or soft-bodied organisms.	<i>Part</i> will be associated with higher sensitivity than <i>All</i>	Rico and Van den Brink, 2015
Feeding guild	<i>Filter-feeder</i> = filters small particles from water <i>Other</i> = primarily an herbivore or scavenger in scraper, grazer, or collector-gatherer feeding guilds <i>Predator</i> = primary feeding guild is predator	Filtering water for small food particles increases exposure to chemicals in the water. Non-predators may also have higher exposure risks to some contaminants due to higher levels in algae and detritus food sources than in prey food sources, and non-predators may be more affected by synergistic effects of toxicity and predation stress.	<i>Filter-feeder</i> will be associated with highest sensitivity and <i>Predator</i> the least	Hartmann and Martin, 1984; Baird and Van den Brink, 2007; Brinke et al., 2010; Ippolito et al., 2012; Rubach et al., 2012; Liess et al., 2017
Habit	<i>Burrower</i> = burrows in substrate <i>Crawler</i> = climbs, crawls, or attaches to substrate/submerged materials <i>Swimmer</i> = swims in water column or skates on water surface	Burrowers and crawlers are exposed to contaminants bound to substrates in addition to contaminants in the water.	<i>Burrower</i> and <i>Crawler</i> will be associated with higher sensitivity than <i>Swimmer</i>	Lange et al., 2014; Berger et al., 2018
	<i>Water</i> = dissolved oxygen acquired from water via gills or cutaneous respiration <i>Atmosphere</i> = at least one aquatic life cycle stage acquires oxygen from atmosphere	Organisms that acquire dissolved oxygen from the water via gills or integument have higher exposure risks to contaminants in the water than organisms that acquire oxygen from the atmosphere.	<i>Water</i> will be associated with higher sensitivity than <i>Atmosphere</i>	Buchwalter et al., 2002; Baird and Van den Brink, 2007; Rico and Van den Brink, 2015; Mondy et al., 2016
	<i>Passive</i> = disperses via wind, drift, or animal vectors <i>Active</i> = disperses via flight, swimming, or crawling	Active dispersers have higher population recovery potential than passive dispersers following exposure at an impacted site due to higher recolonization rates.	<i>Passive</i> will be associated with higher sensitivity than <i>Active</i>	Kefford et al., 2012; Gergs et al., 2016; Mondy et al., 2016
Voltnism	<i>Univoltine</i> = ≤ 1 generation per year <i>Multivoltine</i> = > 1 generation per year	Multivoltine taxa have faster population growth rates, and thus population recovery potential following exposure than taxa that have ≤ 1 generation per year.	<i>Univoltine</i> will be associated with higher sensitivity than <i>Multivoltine</i>	Sherratt et al., 1999; Beketov et al., 2008; Liess and Bakhetov, 2011; Kefford et al., 2012; Lange et al., 2014; Gergs et al., 2016; Mondy et al., 2016



**Fig. 1.** 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 \(2014\)](#).



**Fig. 2.** Examples of two of the 27 sampled farmland drainage ditches.

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.

## 2.2. Study area

Data for this study were collected from 27 agricultural drainage ditches in rural farmland in Eastern Ontario, Canada, across an area of approximately 5000 km<sup>2</sup> in the St. Lawrence watershed of the Mixedwood Plains ecozone (Fig. 1). 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 (City of Ottawa, 2011; DUC, 2010). 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 (City of Ottawa, 2011; DUC, 2010). Networks of open-system constructed drains (drainage ditches) have been established in the area for at least 150 years (Irwin, 1989) and are now ubiquitous features across the region (Fig. 1). 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 al., 2001).

### 2.3. Study sites

Our 27 sampled drainage ditch sites were previously selected for a study investigating relationships between landscape structure and farmland water quality (Collins et al., 2019). Collins et al. (2019) selected ditches in landscapes that represented gradients in landscape variables predicted to influence water quality. They were a minimum of 3 km apart. The 27 study ditches are typical of the farmland drainage ditches across the region (see Fig. 2 for example ditch sites). All ditch sites are exposed, in varying degrees, to agricultural pollution. We emphasize that our goal is to develop an indicator method to distinguish between high vs. low-impacted sites (as opposed to impacted vs. non-impacted sites). Given the context and this objective, including reference sites in our study design was not possible, because there are no completely unimpacted (reference) sites in the study area. Including reference sites from outside the study area would not have been informative, because the drainage ditch sites are different from natural water bodies outside the study area in that they are highly linear water features with unique hydrological characteristics. A comparison of macroinvertebrate traits between ditches and a different type of water body would not tell us whether macroinvertebrate traits can be used to identify more- vs. less-impacted ditch sites. For this objective we needed to compare ditch sites to each other. Conversations with landowners involved in the 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.

A 10-m-long sampling transect was established in the widest accessible section of each ditch for water quality and macroinvertebrate sampling. 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  $22 \pm 11$  SD). Average channel width ranged from 0.88 to 6.27 m (mean  $1.9 \pm 1.1$  SD), and average channel bank height ranged from 0.93 to 3.13 m (mean  $1.7 \pm 0.6$  SD).

### 2.4. Water collection for agrichemical determination

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. We began the first sampling period in early June following an extended period of rainfall (four days) across the study area, to maximize detection of peak agrichemical levels in the drainage ditches. Agrichemical concentrations in farmland surface waters are usually highest this time of year from runoff inputs following planting and post-planting applications and from seed treatments (Hladik et al., 2014; Thurman et al., 1992). Grab samples were collected from the center of each ditch transect and kept in coolers until returned to the laboratory. We used grab samples of the ditch water rather than passive sampling or sediment samples. Passive sampling would have provided time-weighted-average concentrations rather than single-time concentrations (Vrana et al., 2005). However, use of passive sampling in monitoring is limited by a lack of standardized analytical methods, and so grab sampling is still the most widely-used method. We used water samples rather than sediment samples because all compounds assessed in this study are highly soluble.

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 °C, and samples for glyphosate analysis were held in 500 mL plastic bottles and stored at -40 °C 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™ Ion Source, AB Sciex). Details of all analytical methods and quality assurance are provided in Collins et al. (2019). 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 °C until analysis. Nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) was determined by ion chromatography (method 4110C); the limit of detection was 0.1 mg/L (Ontario Ministry of the Environment, 2010).

### 2.5. Aquatic macroinvertebrate sampling

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 Barbour et al., 1999; and U.S. EPA, 1997). We collected invertebrates using a modified dip net measuring 56 cm × 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 using Merritt et al. (2008), Peckarsky (1990), and Pennak (1978).

### 2.6. Are macroinvertebrate traits related to family sensitivities to particular agrichemicals?

We selected seven traits (Table 1) that we predicted would be important determinants of aquatic macroinvertebrate sensitivity to the agrichemicals measured in ditch water (Table 1), and assigned each family a particular state for each trait (Appendix A). 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 A 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”

**Table 2**

Descriptive statistics of agrichemicals measured in water samples collected from 27 drainage ditches during two collection periods in June and July 2014. Atrazine, glyphosate, and three neonicotinoid insecticides were measured in samples from both collection periods and nitrate was measured once in samples collected in July. Total neonicotinoids was the sum of the concentrations of clothianidin, imidacloprid, and thiamethoxam. Overall mean is the mean across all sites and both collection periods. “LOD” = limit of detection; values < LOD were set to LOD/2.

Agrichemical	June range (mean)	July range (mean)	Overall mean	No. sites detected
Nitrate (mg/L)	—	< LOD – 9.0 (2.62)	2.62	26
Atrazine (µg/L)	0.005–2.76 (0.23)	0.005–0.45 (0.1)	0.17	27
Glyphosate (µg/L)	< LOD – 1.82 (0.16)	< LOD – 6.18 (0.61)	0.39	19
Total Neonicotinoids (µg/L)	< LOD – 0.61 (0.04)	< LOD – 0.28 (0.05)	0.04	26
Clothianidin (µg/L)	< LOD – 0.42 (0.03)	< LOD – 0.09 (0.02)	0.02	26
Imidacloprid (µg/L)	< LOD – 0.002 (0.0003)	< LOD – 0.01 (0.002)	0.001	17
Thiamethoxam (µg/L)	< LOD – 0.19 (0.01)	< LOD – 0.23 (0.03)	0.02	26

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 absence/presence 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 analyzed family absence/presence rather than abundance, because absence/presence was more relevant to our goal of developing simple water quality indicators using macroinvertebrate traits. In particular, it should be easier for a practitioner to make an assessment based on the complete presence or absence of a trait category than on the relative abundances of trait categories predicted from models. 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 (as in Quesnelle et al., 2013), an arbitrary criterion aimed at maximizing the number of families with sensitivity estimates (35 families) for the cross-family analyses (below). 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 B), 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 ( $n = 35$  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 using 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).

### 2.7. 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.

### 2.8. 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 (see Section 2.6), 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. This is a more stringent criterion than we used in estimating the family sensitivity coefficients (above). This is because the purpose of the sensitivity coefficients was to produce input values for cross-family analyses, where inferences were made using all 35 estimates together. For those analyses we chose to maximize our sample size, i.e. to maximize the number of families with sensitivity coefficients. In contrast, here our aim was to draw inferences for individual families. We thus used a more stringent criterion for the logistic regressions here, to avoid spurious inferences about individual families.

**Table 3**

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  $> 20\%$  of 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						–	

### 3. Results

#### 3.1. Ditch water quality variables

Descriptive statistics for the agrichemical concentrations measured from the 27 sites are in [Table 2](#), and macroinvertebrate families identified from the sites are listed in [Appendix C](#). 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 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 C](#)).

#### 3.2. Are macroinvertebrate traits related to family sensitivities to particular agrichemicals?

##### 3.2.1. Elimination of correlated traits

We reduced the number of traits from seven to four, based on pairwise dependencies between traits ([Table 3](#); [Appendix D](#)). 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 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 3](#); 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.

##### 3.2.2. 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  $-0.09 \pm 0.70$  SD), atrazine coefficients ranged from -1.51 to 2.87 (mean  $0.16 \pm 0.72$  SD), glyphosate coefficients ranged from -1.56 to 1.59 (mean  $-0.11 \pm 0.76$  SD), and neonicotinoid sensitivity coefficients across the 34 taxa ranged from -3.08 to 3.05 (mean  $-0.07 \pm 1.12$  SD) ([Appendix E](#)). Pairwise correlations across families between the four sensitivity coefficients are shown in [Fig. 3](#). 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.

##### 3.2.3. Relationships between sensitivity coefficients and macroinvertebrate family traits

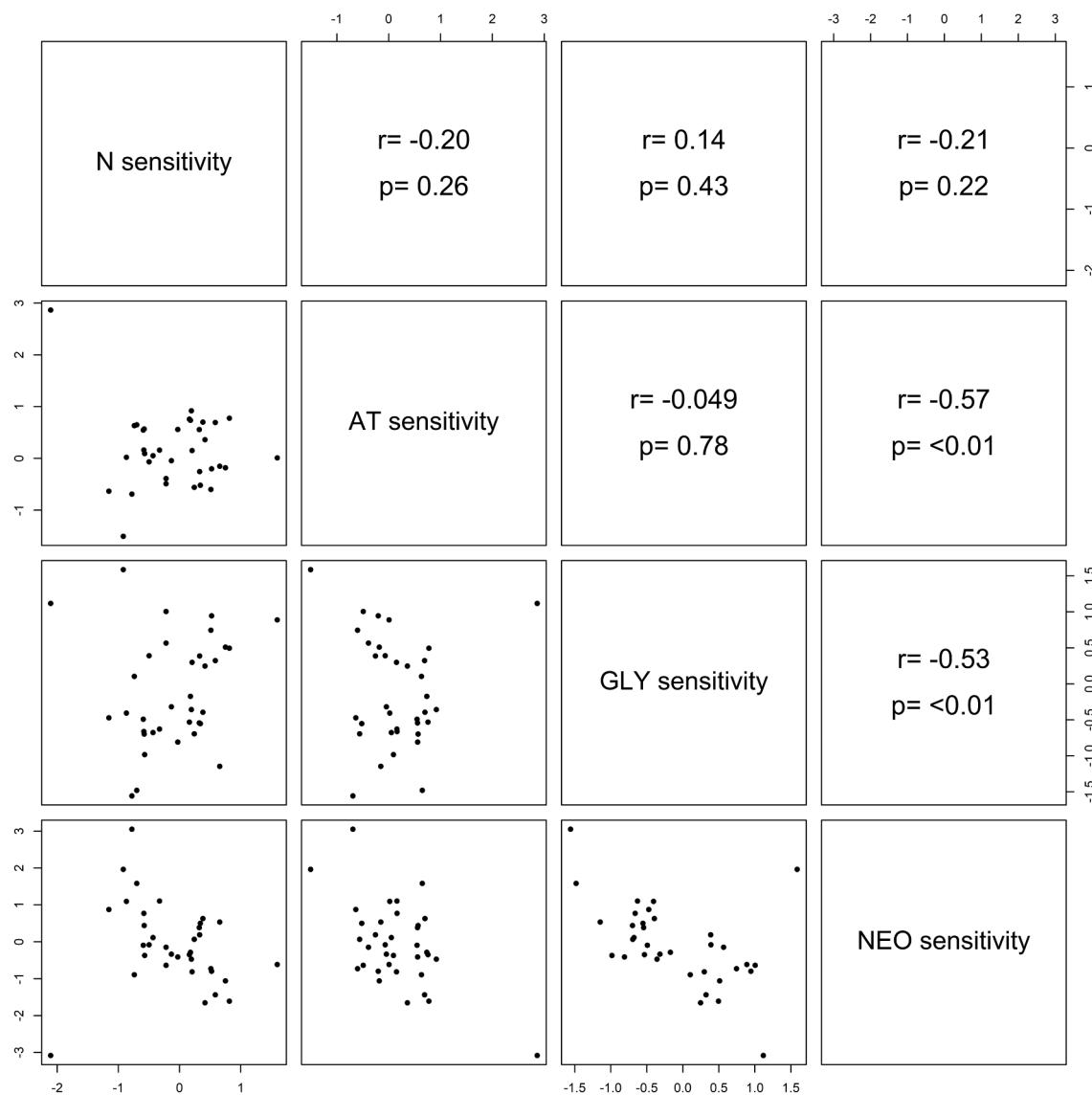
Two traits, oxygen acquisition and dispersal mode, were related to two agrichemical sensitivity coefficients ([Fig. 4](#)). Sensitivity to nitrate was significantly associated with mode of oxygen acquisition, with atmosphere breathers having a higher mean sensitivity coefficient than water breathers ( $t = -2.31$ ,  $p = 0.03$ ,  $df = 30$ ). Glyphosate sensitivity was significantly related to dispersal mode, with passive dispersers having a higher mean sensitivity coefficient than active dispersers ( $t = 2.10$ ,  $p = 0.04$ ,  $df = 30$ ).

#### 3.3. 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 ([Figs. 5 and 6](#)). 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 ([Fig. 4a](#)), and the mean standardized glyphosate coefficient for passive dispersers was higher than the mean standardized coefficient for families that actively disperse ([Fig. 4b](#)), 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 nitrate levels ([Fig. 5](#)), and three passive dispersers (Sphaeriidae, Valvatidae, and Hyalellidae) had negative glyphosate sensitivity coefficients ([Fig. 6](#)). 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.

#### 3.4. 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 E](#)). 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 ([Fig. 7](#)). However, Asellidae was relatively common across the study sites, with only four absences, and Hydropsychidae was rare, with only three presences ([Fig. 7](#)). 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



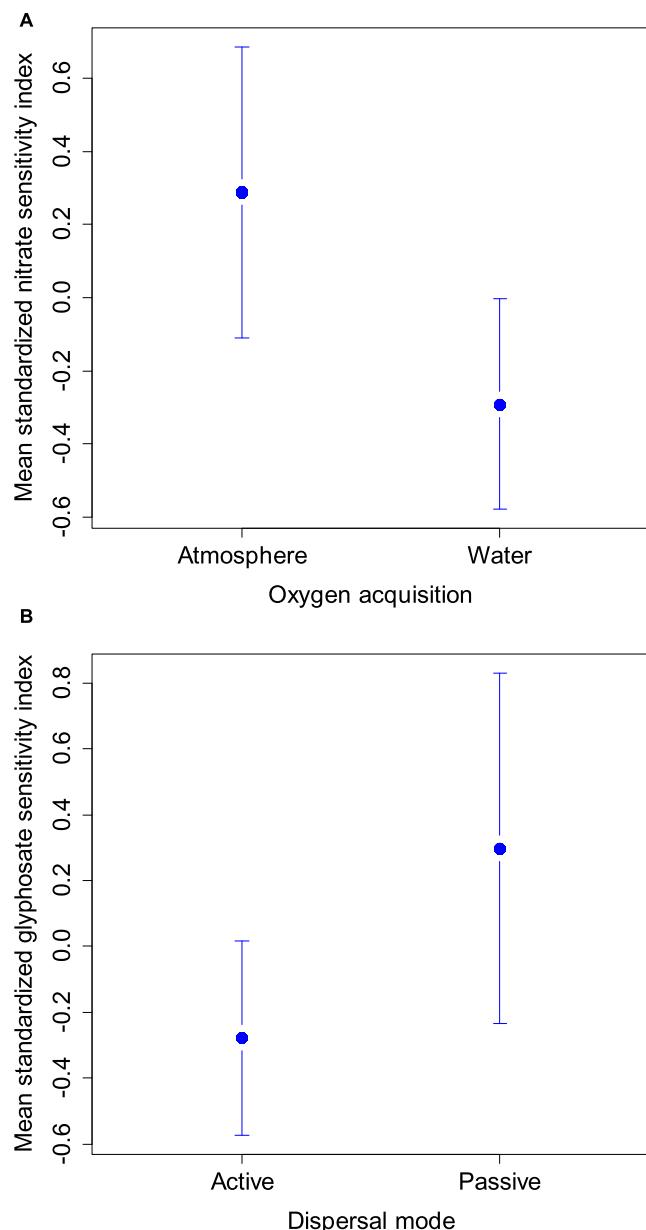
**Fig. 3.** Cross-family pairwise correlations of sensitivities to four agrichemicals. Sensitivities are standardized coefficients from binomial generalized linear models of family absence/presence on concentrations of nitrate (N), atrazine (AT), glyphosate (GLY), and neonicotinoid insecticides (NEO).

representation of absences and presences (33% and 67%, 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 ( $LOD/2 = 0.05 \text{ mg/L}$ ), 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. 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 (Fig. 4). The relationship between dispersal mode and glyphosate sensitivity supports the prediction that passive dispersers are more sensitive, at least

to glyphosate pollution, than active dispersers, suggesting that taxa with active dispersal modes have higher population recovery rates than passive dispersers due to higher immigration rates. 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,



**Fig. 4.** 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 higher mean sensitivity coefficients 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 higher mean sensitivity coefficients to glyphosate concentration, i.e. a higher probability of absence with increasing glyphosate levels, than families that disperse actively.

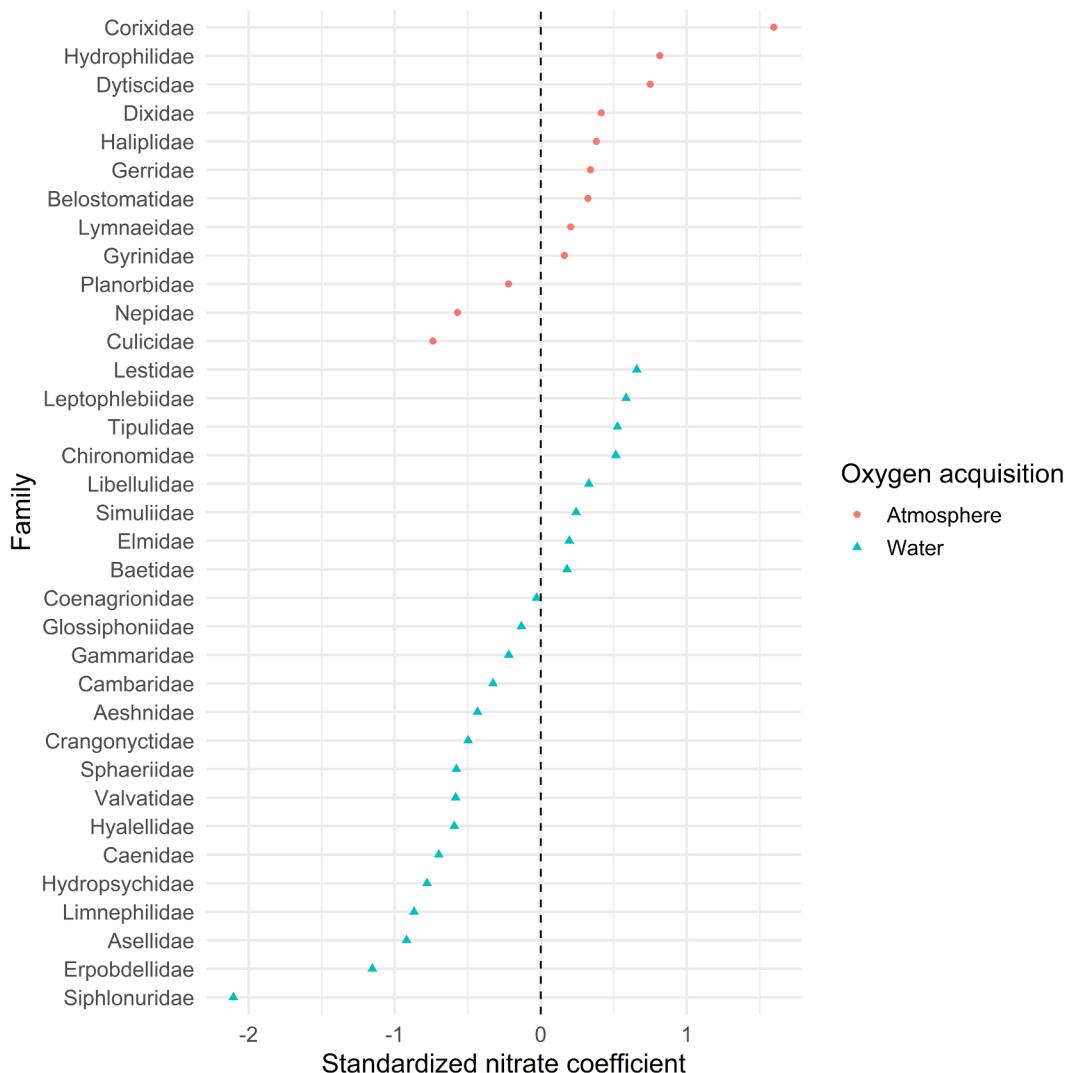
Berger et al. (2018) found similar unexpected relationships between plastron respiration (a type of air-bubble breathing) and sensitivity to water chemistry variables, including nitrate. While respiratory bubbles are initially acquired from the atmosphere to serve as underwater oxygen supplies, they 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 F).

Although we detected relationships between traits and sensitivity to chemical contamination, these relationships are not consistent enough to develop reliable bioindicators (Figs. 5 and 6). 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 absence or presence 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 a 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 3), 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 D). 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.

Our small sample of 27 sites, for estimating the family sensitivity coefficients, may have limited our ability to detect consistent relationships between traits and sensitivities. While this small sample size does not bias the sensitivity estimates, it does increase our uncertainty in them (Smith et al., 2009). Thus, some estimates are likely larger and others are likely smaller than the actual sensitivities. On the other hand, multiple families were included in each of the trait categories for the trait-sensitivity analyses, so these estimation errors were likely averaged out to some extent. Nevertheless, it remains possible that a larger number of sites might have revealed stronger trait-sensitivity relationships. Thus, it would be worthwhile for future research to re-examine these relationships using larger data sets.

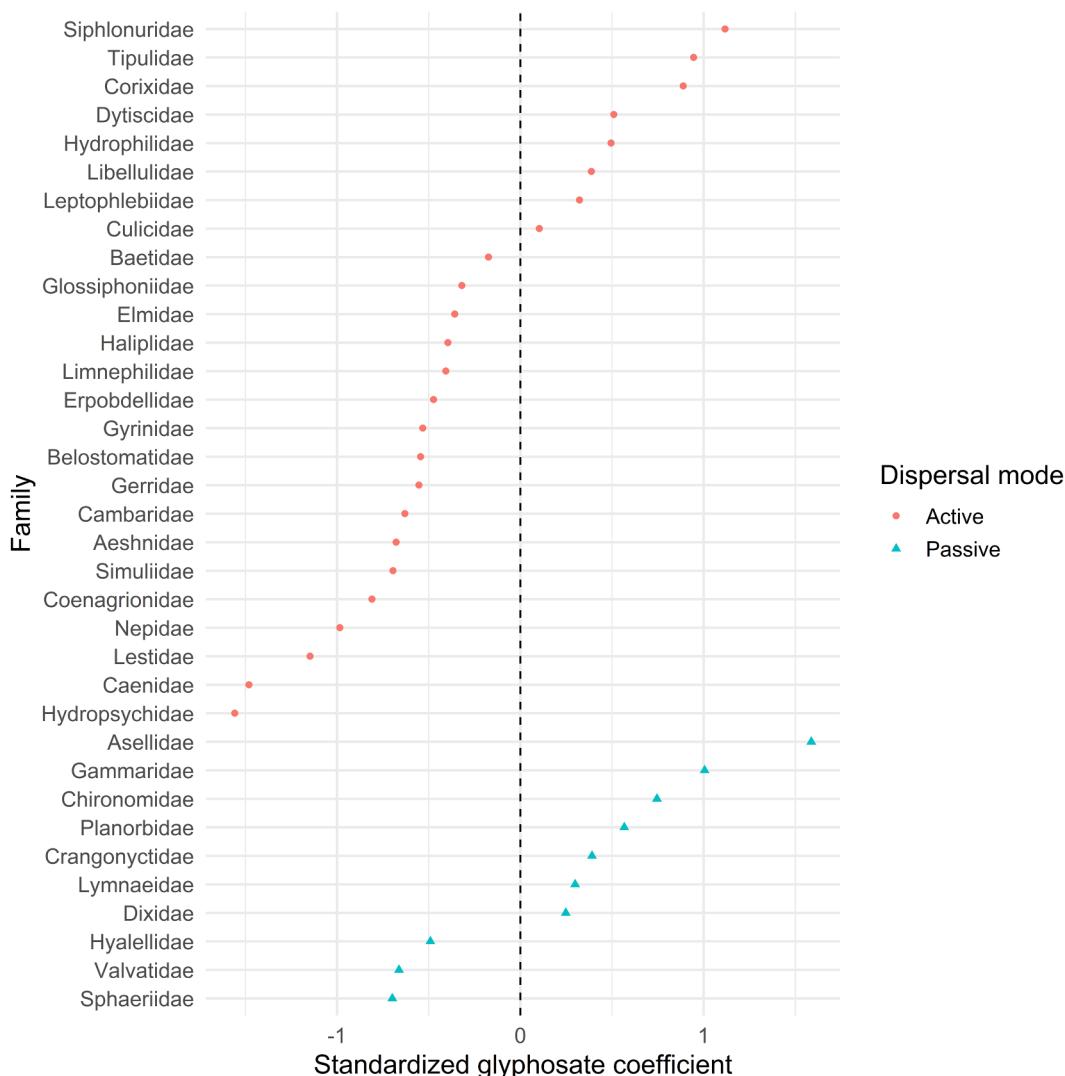


**Fig. 5.** Standardized nitrate coefficients from each macroinvertebrate family binomial generalized linear model, showing families that acquire oxygen from the atmosphere vs. from the water. While the mean nitrate coefficient for atmospheric-breathing families is higher than the mean coefficient for families that acquire oxygen from the water (Fig. 4a), 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.

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 a lack or low levels of particular chemicals. Our post-hoc analysis suggests that the presence of Corixidae may indicate low ditch nitrate levels (Fig. 7; Appendix E). 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. This was not true when we used 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 absence might be useful 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.,



**Fig. 6.** 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 (Fig. 4b), 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.

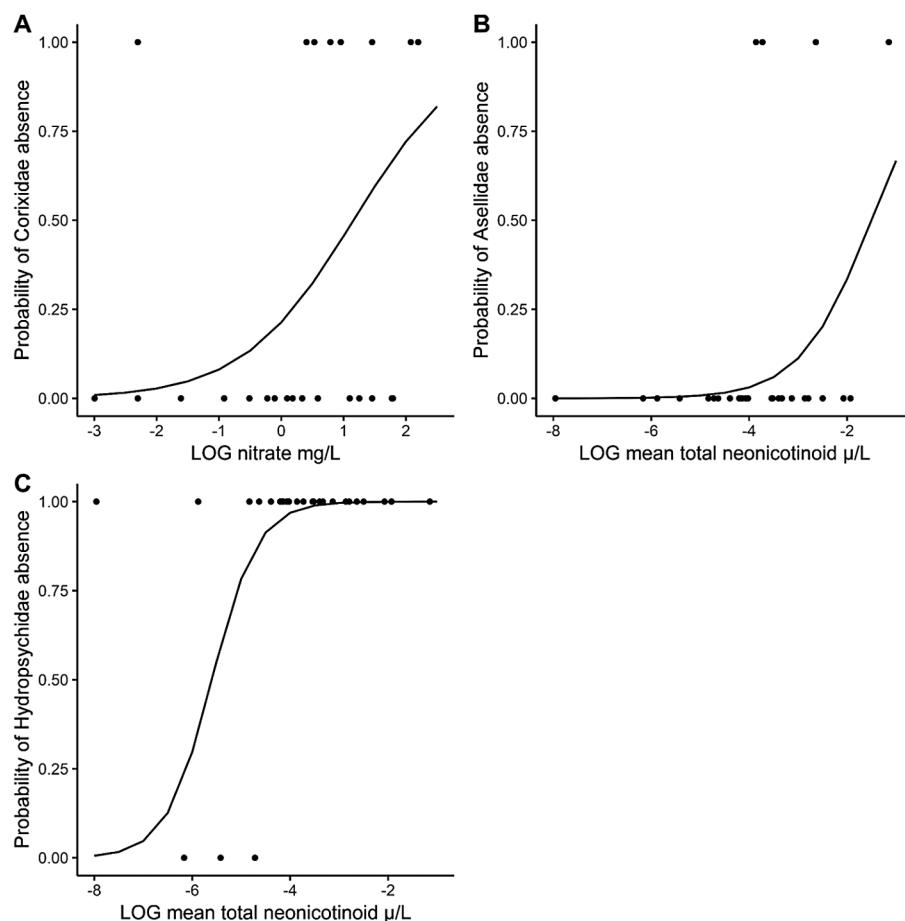
1999; Melzer, 1999; Potapova and Charles, 2007; Resh, 2008). Plants, in particular, have been suggested to be good indicators of eutrophic conditions (Melzer, 1999; Penning et al., 2008). In our study area, aquatic plant communities were previously shown to be negatively affected by nitrate levels while 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 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).

## 5. Conclusions

We used a novel approach to assess the potential for macroinvertebrate family traits to be indicators of specific agrichemicals in farmland drainage ditches. Agrichemicals were detected in water samples collected from every ditch site in varying concentrations, with levels of some contaminants exceeding Canadian water quality guidelines for the protection of aquatic life (CCME, 1999, 2007, 2012). Our



**Fig. 7.** 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.

study revealed two relationships between traits and macroinvertebrate chemical sensitivities: passive dispersers were more sensitive to glyphosate than active dispersers, and atmosphere breathers were more sensitive to nitrate than dissolved-oxygen breathers. However, these trait-sensitivity relationships lacked enough consistency to be used as simple, reliable indicators of these contaminants. Alternatively, a post-hoc evaluation suggested that certain taxa may be indicative of low vs. high levels of particular agrichemicals. In particular, the hemipteran family Corixidae appears to be indicative of nitrate levels. The unexpected negative relationship between atmosphere breathers, including Corixidae, to nitrate levels may be driven by the type of atmospheric-breathing strategy; in particular, respiratory bubble-breathers may be more sensitive than other types of atmosphere breathers. Our study provides new insights on the responses of macroinvertebrate communities to elevated levels of agricultural pollutants in farmland water bodies. However, in the context of our overall goal of identifying simple, easy-to-use, trait-based indicators to distinguish between highly-impacted vs. low-impacted sites, our results suggest that invertebrate indicators are unlikely to be more efficient than chemical sampling.

#### CRediT authorship contribution statement

**Sara J. Collins:** Conceptualization, Methodology, Formal analysis,

Investigation, Writing - original draft, Visualization. **Lenore Fahrig:** Conceptualization, Methodology, Writing - review & editing, Supervision.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

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## Appendices

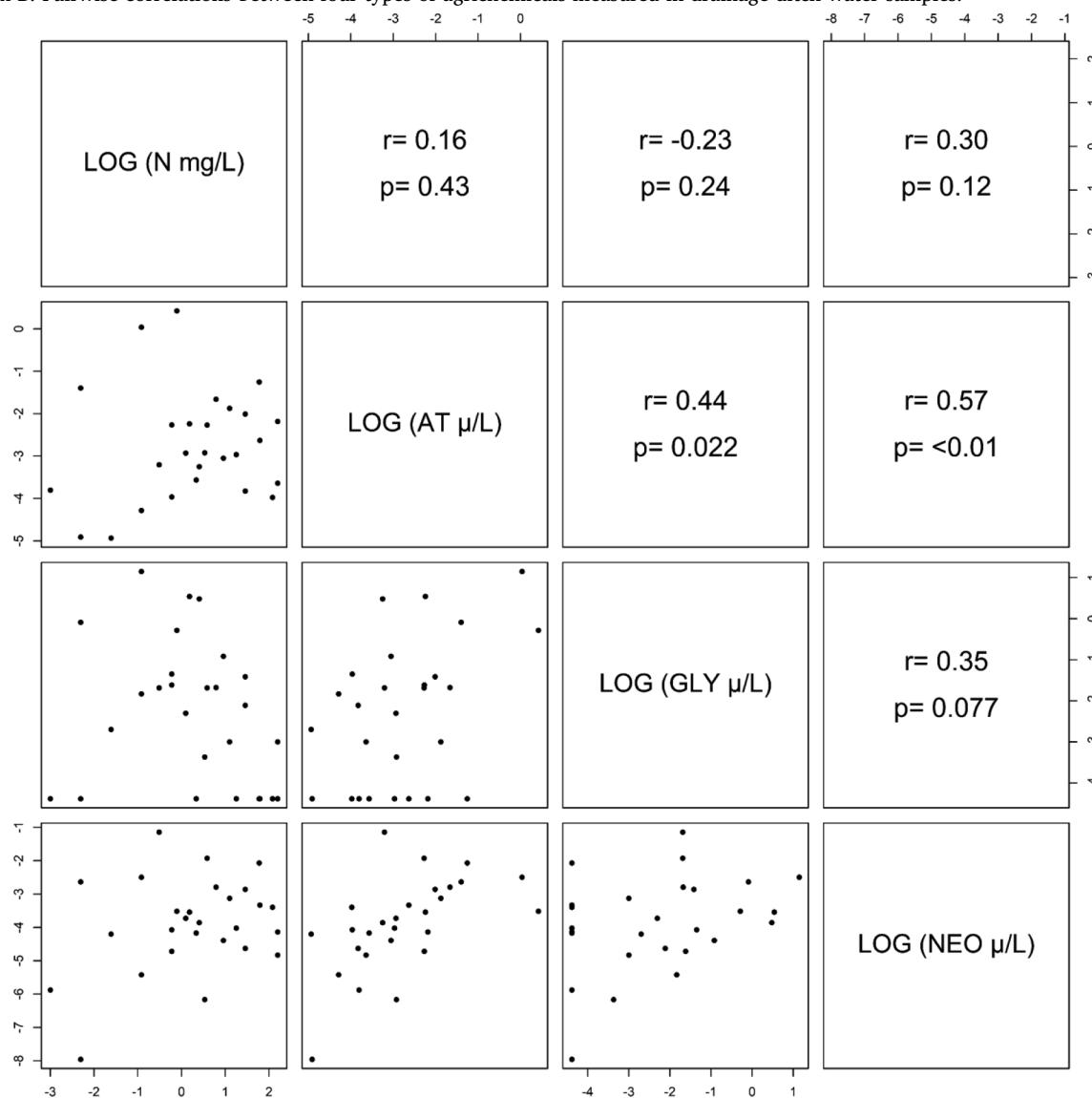
Appendix A. Trait state assignments for 7 traits (body size, degree of body armouring, feeding guild, habit, oxygen acquisition, dispersal mode, and voltinism; see descriptions in Table 1) to 35 aquatic macroinvertebrate families collected from 27 agricultural drainage ditches in Eastern Ontario. Source 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
	Lymnaeidae	large	part	other	crawl	atmosphere	passive	uni	1, 3, 11
Basommatophora	Planorbidae	small	part	other	crawl	atmosphere	passive	multi	1, 3
	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
Decapoda	Hydrophilidae	small	all	other	crawl	atmosphere	active	uni	1, 3
	Cambaridae	large	all	other	crawl	water	active	uni	1, 4, 5, 6
	Chironomidae	small	part	other	crawl	water	passive	multi	1, 7
	Culicidae	small	part	other	crawl	atmosphere	active	multi	1, 3
Diptera	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
	Baetidae	small	part	other	swim	water	active	multi	1, 3
Ephemeroptera	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
	Belostomatidae	large	part	predator	crawl	atmosphere	active	multi	1, 3
Hemiptera	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
Heterostrophida	Valvatidae	small	part	other	crawl	water	passive	multi	1, 9
	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
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

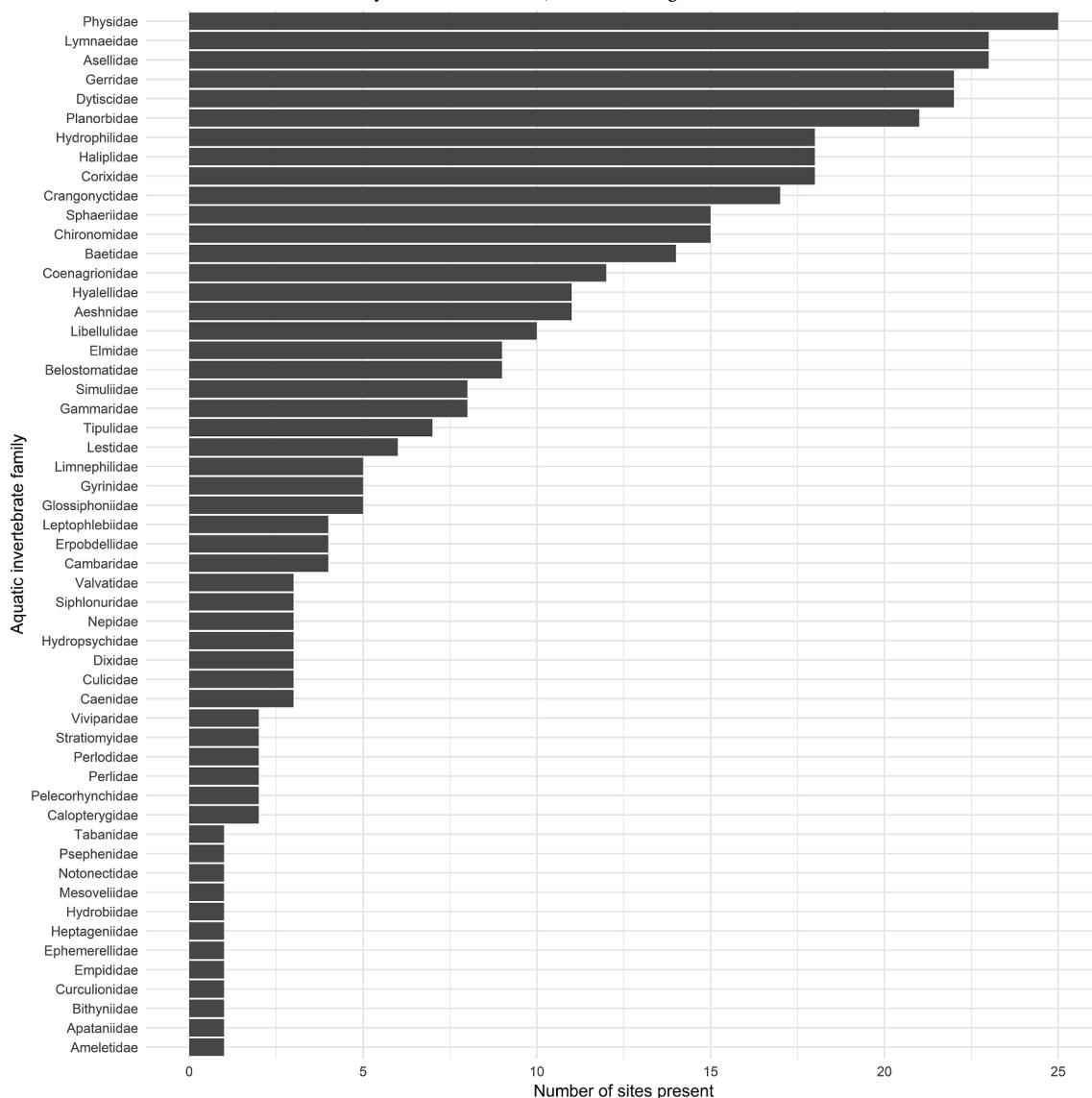
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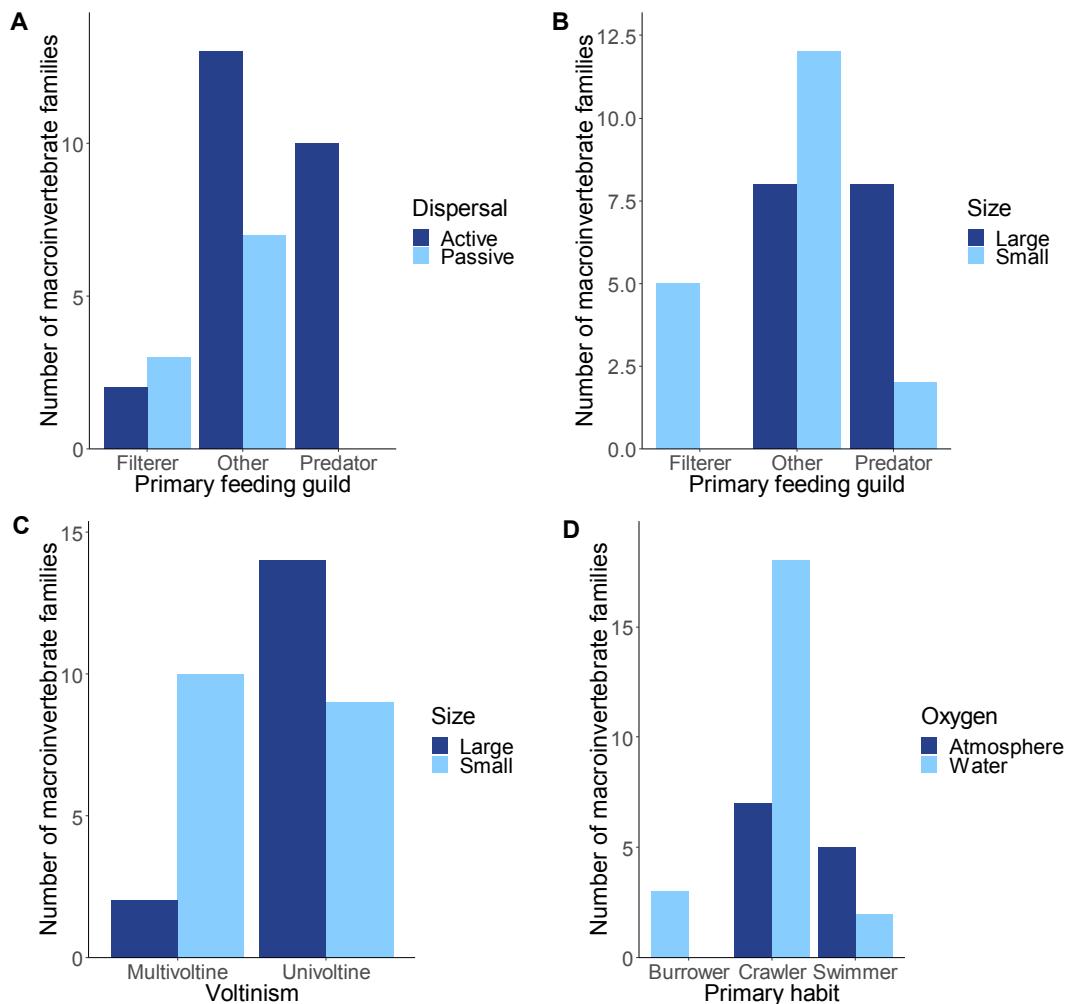
Appendix B. Pairwise correlations between four types of agrichemicals measured in drainage ditch water samples.



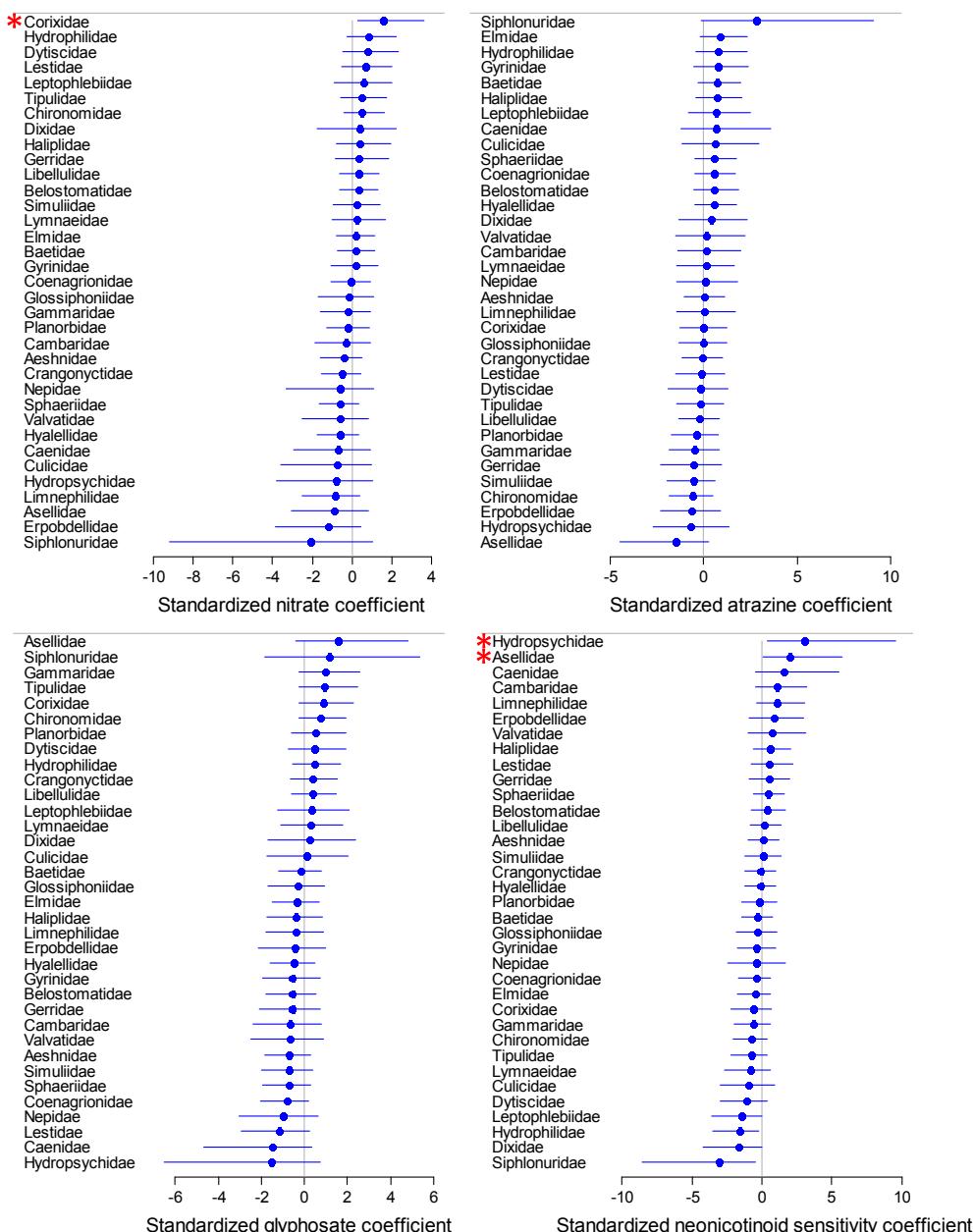
Appendix C. The 54 aquatic macroinvertebrate families found in samples collected from 27 agricultural drainage ditches in Eastern Ontario, Canada, and the number of sites in which each family was encountered, in decreasing order.



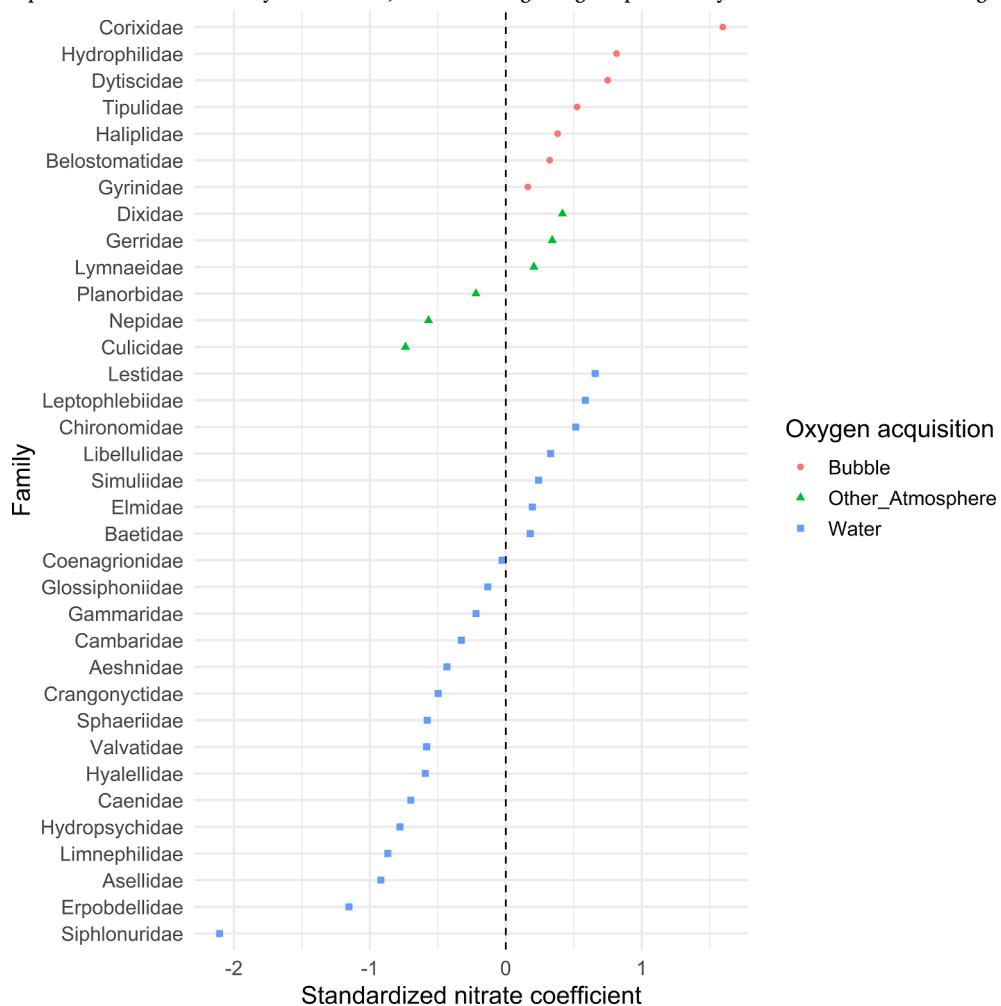
Appendix D. Significant pairwise dependencies between macroinvertebrate family traits from 35 families collected from agricultural drainage ditches. (a) A significant relationship between feeding guild and dispersal mode, driven by all predators being active dispersers. (b) A significant relationship between feeding guild and body size, driven by all filterers 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 E. 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).



Appendix F. 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.



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