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Macrophytes and Atrazine in Ponds of Southwest Missouri

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MACROPHYTES AND ATRAZINE IN PONDS OF SOUTHWEST MISSOURI

A Masters Thesis

Presented to

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In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Christine Michelle Cornish

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Biology

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ABSTRACT

Wetlands are often the ultimate destination of agrochemicals. The increased use of these pollutants has resulted in their increased transport, via runoff and spray drift, into wetlands. Atrazine, a commonly used herbicide, has been detected in surface water, groundwater, soil, and sediment, and has shown to have adverse impacts on aquatic biota, such as fish and amphibians. Few studies have reported on the relationships between atrazine and macrophytes. I measured atrazine concentrations in surface sediments in agricultural, conservation, and golf course ponds of southwest Missouri, and investigated how those concentrations might be related to macrophyte communities, and pond environmental characteristics. My study found that water pH, depth, open water area, and sediment organic matter content varied among ponds; while macrophyte cover, richness, water conductivity, atrazine concentrations, and sediment particle size were similar. Water pH and sediment organic matter content were found to be significant predictors of macrophyte composition and frequency, explaining approximately 27% of the variation. Water depth and open water area were found to be significant predictors of macrophyte presence-absence, explaining 25% of the variation. *Lemna minor* and *Spirodela polyrhiza* were found to be significant indicator species of conservation ponds; whereas *Spirogyra* spp. was a significant indicator species of golf course ponds.

KEYWORDS: wetland ecology, ecotoxicology, ponds, macrophytes, atrazine

This abstract is approved as to form and content

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Dr. La Toya Kissoon-Charles
Chairperson, Advisory Committee
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Approved:

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Dr. La Toya Kissoon-Charles

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I dedicate this thesis to Dr. Kissoon-Charles as my advisor, role model, and a great woman of science.

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INTRODUCTION

Wetlands are often considered ecotones, transitional zones between terrestrial and aquatic ecosystems that intercept runoff from surrounding land, and aids in the removal of chemicals and pollutants (Risser 1995). These ecosystems, which include tidal (e.g mangrove swamp) and freshwater (e.g. inland marsh or agricultural pond) wetlands (Mitsch and Gosselink 2015), can serve as sinks and/or transformers of many incoming chemicals and pollutants (Chayapan et al. 2015) through various biogeochemical processes. These processes are influenced by the wetland's hydrologic conditions, physiochemical properties (e.g., hydric soils, redox status), and biota (specifically, macrophytes and microorganisms) (Mitsch and Gosselink 2015). Some of the chemicals and pollutants that enter wetlands such as metals and pesticides, persist in the environment, remain untransformed, and can cause adverse effects to wetland biota (Brönmark and Hansson 2002). Consequently, wetlands are considered highly vulnerable ecosystems (Dudgeon et al. 2006), as they are often the ultimate destination of terrestrially applied chemicals (Yan et al. 2016). Macrophytes in particular, can be subjected to various herbicides through their leaves and roots as they can be in direct contact with chemicals in the water or sediments (Heegaard et al. 2001). Many herbicide compounds can settle and/or accumulate in wetland sediments, potentially disrupting the wetland's natural biological functions (Crafter et al. 1992). For example, due to heavy annual applications (Merini et al. 2009), atrazine is of particular concern to aquatic ecosystems. The objectives of my research included determining the following in agricultural, conservation, and golf course ponds; (1) macrophyte composition and

frequency; (2) sediment atrazine (herbicide) concentrations; (3) chemical and physical water and sediment characteristics; and (4) relationships between macrophyte composition and frequency, sediment atrazine concentrations, and chemical and physical water and sediment characteristics.

Macrophytes: Effects on Pollutants in Wetlands

Macrophytes play crucial roles in wetlands, including stabilizing sediment, assimilating and cycling nutrients, capturing and transforming pollutants, providing habitat and food for other organisms (Cedergreen et al. 2005), and improving water quality (Scheffer and Jeppesen 1998; Dodds and Whiles 2010). The aquatic flora of wetlands includes emergent, floating, and submerged species, which all contribute to a wetland's ability to serve as a sink and/or transformer of chemicals. Macrophytes have several special adaptations to tolerate inundated conditions they experience in wetlands (Cronk and Fennessy 2001). One such adaptation is the development of aerenchyma tissue, which allows for sufficient oxygen diffusion from the shoots to the roots (Yang et al. 2017). Oxygen diffusion to the roots can oxygenate the sediment via radial oxygen loss (ROL), and thus affect the redox status of the rhizosphere (Sand-Jensen et al. 1982). For example, *Isoetes lacustris*, *Littorella uniflora*, *Lobelia dortmanna*, *Myriophyllum spicatum*, *Potamogeton crispus*, *Potamogeton friesii*, *Potamogeton pectinatus,* and *Sparganium simplex* were reported to have a high sediment oxygenating capacity (Mermillod-Blondin et al. 2008; Sand-Jensen et al. 1982). This adaptation creates an oxidized-reduced interface, which heavily influences biogeochemical cycling, and pollutant transformations in wetlands (Mann and Wetzel 2000; Mermillod-Blondin et al.

2008). Yang et al. (2017) found a positive correlation between ROL and metal adsorption to the root surface and in the rhizosphere of several emergent macrophytes, including *Acorus tatarinowii*, *Alocasia cucullata*, *Cyperus alternifolius*, *Echinodorus amazonicus*, *Echinodorus baothii*, *Eleocharis geniculata, Hydrocotyle vulgaris*, *Panicum repens, Scirpus triqueter*, and *Veronica serpyllifolia*. This finding indicated that ROL and subsequent oxidation of the rhizosphere can affect metal mobility, thus bioavailability.

The ability to oxidize the rhizosphere, along with other attributes such as high biomass and tolerance of various pollutants, make many macrophytes ideal candidates for phytoremediation (Yoshida et al. 2006; Williams 2010; Chayapan et al. 2015). Phytoremediation involves the use of plants to remove, transform, or degrade environmental pollutants (Wu et al. 2004; Ali et al., 2013; Moreira et al. 2013; Chayapan et al. 2015) such as nitrogen, phosphorus, metals, pharmaceuticals, and pesticides. Numerous studies have examined the efficacy of this remediation technique for removal of various pollutants in contaminated waters and sediments (Cejudo-Espinosa et al. 2009; Marecik et al. 2011; Wang et al. 2012; Farid et al. 2014).

Several macrophytes have been found to effectively and efficiently remove chemicals and pollutants from contaminated water and sediment. For example, *Typha* and *Phragmites* are emergent macrophytes having high biomass that easily take up phosphorous from sediments, temporarily preventing this element from re-entering the water column (Mackie 2004). Additionally the macrophytes, *Brassica juncea*, *Colocasia esculenta*, *Cyperus malaccensis*, and *Typha angustifolia* were reported to be effective accumulators of metals such as cadmium, copper, lead, and zinc (Cd, Cu, Pb, and Zn) (Wu et al. 2004; Chayapan et al. 2015). Algae such as *Chlorella kessleri*, *Chlorella*

sorokiniana, *Chlorella vulgaris*, and *Scenedesmus obliquus* were also found to be effective accumulators of metals (Cd, Cu, and Zn), and wastewater contaminants including, nitrogen (N) and phosphorus (P) (Yoshida et al. 2006; Arbib et al. 2014). Zhang et al. (2013) used *Scirpus validus* to remove pharmaceuticals in hydroponic mesocosms, including caffeine, diclofenac, and naproxen from solution.

Several species of macrophytes have also been reported to accelerate herbicide degradation and removal from wetlands, thus minimizing its distribution in the environment. For example, Rice et al. (1997) investigated the effectiveness of three macrophytes (*Ceratophyllum demersum, Elodea canadensis,* and *Lemna minor*) to remove atrazine from water. They found that all vegetated systems, specifically, *C. demersum* significantly lowered atrazine's half-life and removed the highest percentage of atrazine, compared to non-vegetated systems (Table 1). Atrazine can have a low degradation rate when using physicochemical techniques, however microbial degradation plays an important role by more efficiently degrading this compound (Singh and Jauhari 2017). Previous studies reported that various pesticides had decreased persistence and/or higher removal rates in the presence of macrophyte-microbial activity (Pritchard et al. 1985; Kruger et al. 1997; Rupassara et al. 2002; Sun et al. 2004; Iker et al. 2010). For example, Wang et al. (2012) investigated atrazine removal using three macrophytes (*Acorus calamus*, *Iris pseudocorus*, and *Lythrum salicaria*) in hydroponic systems under sterile and non-sterile conditions. They reported that after 20 days of exposure, atrazine removal increased and half-life decreased in the presence of macrophytes, and both removal and half-life were most affected under non-sterile conditions when macrophytes and an abundance of microorganisms were present (Table 1). These studies all emphasize

the important role macrophytes play in the remediation of various pollutants. These important functions provided by wetlands, macrophytes, and microorganisms could continue to be advantageous for remediating agrochemicals deposited in wetlands.

Terrestrially Applied Herbicides: Effects on Biota

Agrochemical usage has increased exponentially over the last six decades (Benton et al. 2003). It has long been hypothesized that this increased usage results in increased transport of agrochemicals into wetlands (Correll and Wu 1982). According to the United States Department of Agriculture (USDA), approximately 45% of land in the United States has dominant agricultural land use practices, and approximately 115.5 million hectares of that land is periodically treated with herbicides (USDA 2014). This extensive use has led to their spread in the environment. Terrestrially applied herbicides, such as atrazine, are primarily transported into wetlands via runoff and spray drift, and have been detected in surface water, groundwater (Detenbeck et al. 1996), soils, and sediments (Kruger et al. 1997; Vonberg et al. 2014).

In this study, I focused on atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-traizine), a selective, broad-leaf, triazine herbicide, that targets unwanted plant species by inhibiting photosynthesis. It is a commonly used herbicide, which was first registered in 1958 (Guo et al. 2016), and is predominantly used in agriculture on corn, sorghum, and sugarcane croplands (Figure 1), as well as turf grasses like residential lawns and golf courses (EPA 2017). It has been detected up to twenty times more frequently in groundwater than any other herbicide (Graymore et al. 2001). Atrazine is a persistent organic pollutant (POP), which means that it has the ability to remain in the environment

for prolonged periods of time (half-life in surface soil, 77-101 days; half-life in subsurface soil, >900 days) (Blume et al. 2004). It is a relatively water soluble (33 μg mL^{-1} at 22 °C) (Solomon et al. 1996) and mobile compound (Howard 1991), which enables easy transport and distribution throughout the environment.

The United States Environmental Protection Agency (US EPA) has classified atrazine as a restricted use herbicide, only allowing use by certified applicators (EPA 2017). Atrazine is also under continuous assessment through Atrazine Monitoring Programs (AMPs) administered by the US EPA to determine potential human and/or environmental risks. Atrazine concentrations in drinking water are measured weekly during peak application season (May – June), and bi-weekly for the rest of the year; whereas concentrations in streams and watersheds are measured every 60 days (EPA 2017). Additionally, a maximum concentration of 10 ng mL^{-1} has been established as non-detrimental to macrophyte communities (EPA 2017). However, atrazine toxicity monitoring on macrophytes has varied widely due to uncertainties such as types of macrophytes commonly exposed to atrazine, and unknown toxicity/sensitivity limits of common macrophyte test species (Dobbins et al. 2010). Though limited data are available for macrophytes, potential impacts on other organisms have been more extensively researched.

Available data indicates that atrazine may produce negative consequences for all trophic levels, in addition to plants. Previous research has reported adverse effects of atrazine on various organisms dating back to 1963, when Ashton et al. (1963) studied the effects of 10 mg L^{-1} atrazine on the chloroplasts of *Phaseolus vulgaris* (red kidney bean). They found atrazine caused histological defects after 30 hours of exposure, such as grana

swelling and deconstruction or breakage of chloroplast membranes (Ashton et al. 1963). Dewey (1986) found that atrazine had direct and indirect effects on zooplankton by reducing their offspring production, and depleting their food source by decreasing algal populations. Invertebrates, including aquatic insects, are also indirectly impacted by atrazine, due to decreasing algae and macrophyte populations, which reduces food and habitat availability (Macek et al. 1976; Detenbeck et al. 1996; Gruessner and Watzin 1996). Research conducted on several fish and amphibian species exposed to different concentrations of atrazine (0.03 mg L^{-1} – 0.50 mg L^{-1}) showed various behavioral and developmental effects, such as erratic swimming patterns, reduced growth rates, and gill and liver lesions (Steinberg et al. 1995; Alazemi et al. 1996; Plhalova et al. 2012). Atrazine has also been reported to have various physiological and morphological effects in various species of frogs throughout sexual development at concentrations as low as 0.01 μg L^{-1} (Hayes et al. 2002; 2003; 2006; 2010; 2011). These studies linked atrazine exposure to significant hormonal imbalances, which ultimately led to its label as an endocrine disruptor (Hayes et al. 2002). Morphological changes have also been observed in studies involving mammals exposed to atrazine. For example, chromosomal abnormalities were observed in the ovary cells of hamsters after 48 hours of exposure at levels (< 3 μg L^{-1}) deemed safe by the US EPA for drinking water (Newman 1995; Biradar and Rayburn 1995). Atrazine has also been implicated as a possible human carcinogen (Jowa and Howd 2011). These studies emphasize the comprehensive research that has been conducted on the adverse effects of atrazine on aquatic animals, however few studies have reported effects on macrophyte communities.

Correll and Wu (1982) investigated atrazine toxicity on submerged macrophytes after an observed decline in aquatic plant populations in estuarine and marine habitats. Their microcosm experiments showed photosynthesis inhibition in *Potamogeton pectinatus* and *Zoster marina* when exposed to 650 μg L^{-1} atrazine, and varying percentages of mortality in *Vallisneria americana* when exposed to 12-120 μg L^{-1} atrazine. Jones and Winchell (1984) also examined the effects of atrazine on photosynthesis in *Myriophyllum spicatum, Potamogeton perfoliatus*, *Ruppia maritime*, and *Zannichellia palustris*. They found that at average concentrations of 20 μg L^{-1} atrazine, photosynthesis was inhibited by 1%, and at about 95 μg L^{-1} atrazine, photosynthesis was inhibited by 50%. Another study using *Myriophyllum spicatum* found decreases in branch development after 5 days of exposure to atrazine concentrations less than 100 mg L^{-1} (Christopher and Bird 1992). These studies collectively demonstrate that atrazine entering wetlands can have potentially detrimental concentrations, particularly at levels above the EPA drinking water limit (3 μg L^{-1}) (EPA 2017).

Objectives of My Research

The objectives of my study were to (1) determine macrophyte composition and frequency among ponds in the vicinity of agricultural, conservation, and golf course areas; (2) investigate how sediment atrazine concentrations varied among these ponds; (3) investigate how water and sediment characteristics varied among these ponds; and (3) examine relationships between macrophyte community variables (composition, frequency, and presence-absence), sediment atrazine concentrations, and environmental variables (water depth, open water area, pH, and conductivity; sediment organic matter

content, and particle size). Few studies have reported atrazine concentrations in wetland ecosystems, have investigated relationships between atrazine concentrations and macrophyte abundance, or have investigated relationships between multiple environmental characteristics (water and sediment), atrazine concentrations, and macrophyte abundance. Results from multiple studies have found relationships between single variables (Figure 2). However, my research intends to fill the gap in the literature by researching relationships between multiple variables, including macrophyte abundance, sediment atrazine concentrations, and water and sediment characteristics in ponds. I hypothesized that atrazine concentrations would vary based on surrounding land use (conservation < agricultural < golf course), and that low macrophyte communities (composition, frequency, and richness) would be associated with higher atrazine concentrations (Figure 2).

Table 1. Reported half-life and removal of atrazine in contaminated water in systems with and without macrophytes.

Macrophyte	**Condition**	**Atrazine half-life (days)**	**Removal (%)**	**Reference**
No macrophytes		144	15	Rice et al. 1997
Ceratophyllum demersum		12	58.7	
Elodea canadensis		25	36.8	
Lemna minor		78	15	
No macrophytes	sterile	24.2 ± 0.7	41.4 ± 1.7	Wang et al. 2012
	non-sterile	20.0 ± 1.5	47.6 ± 1.8	
Acorus calamus	sterile	6.1 ± 0.5	88.2 ± 2.4	
	non-sterile	5.1 ± 0.2	93.3 ± 1.2	
Iris pseudocorus	sterile	4.9 ± 0.2	96.8 ± 0.6	
	non-sterile	4.6 ± 0.3	97.2 ± 0.2	
Lythrum salicaria	sterile	6.3 ± 0.5	92.7 ± 1.1	
	non-sterile	5.6 ± 0.2	94.4 ± 0.4	

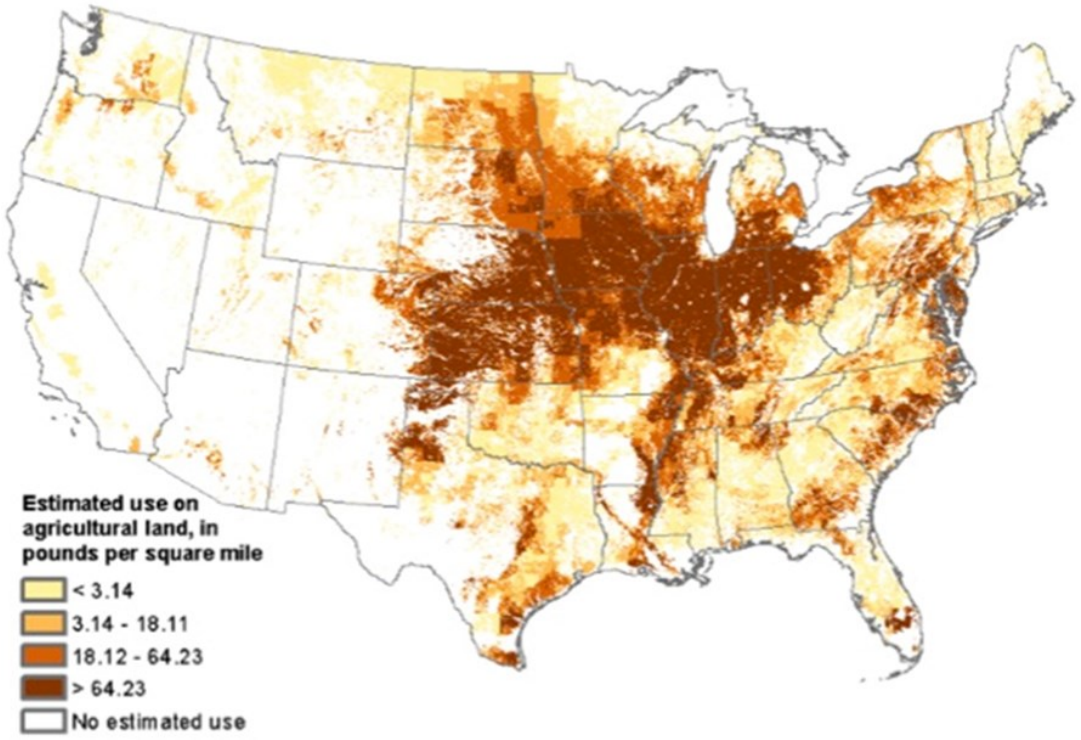

Figure 1. Estimated agricultural use of atrazine in the United States in 2015 (USGS 2015).

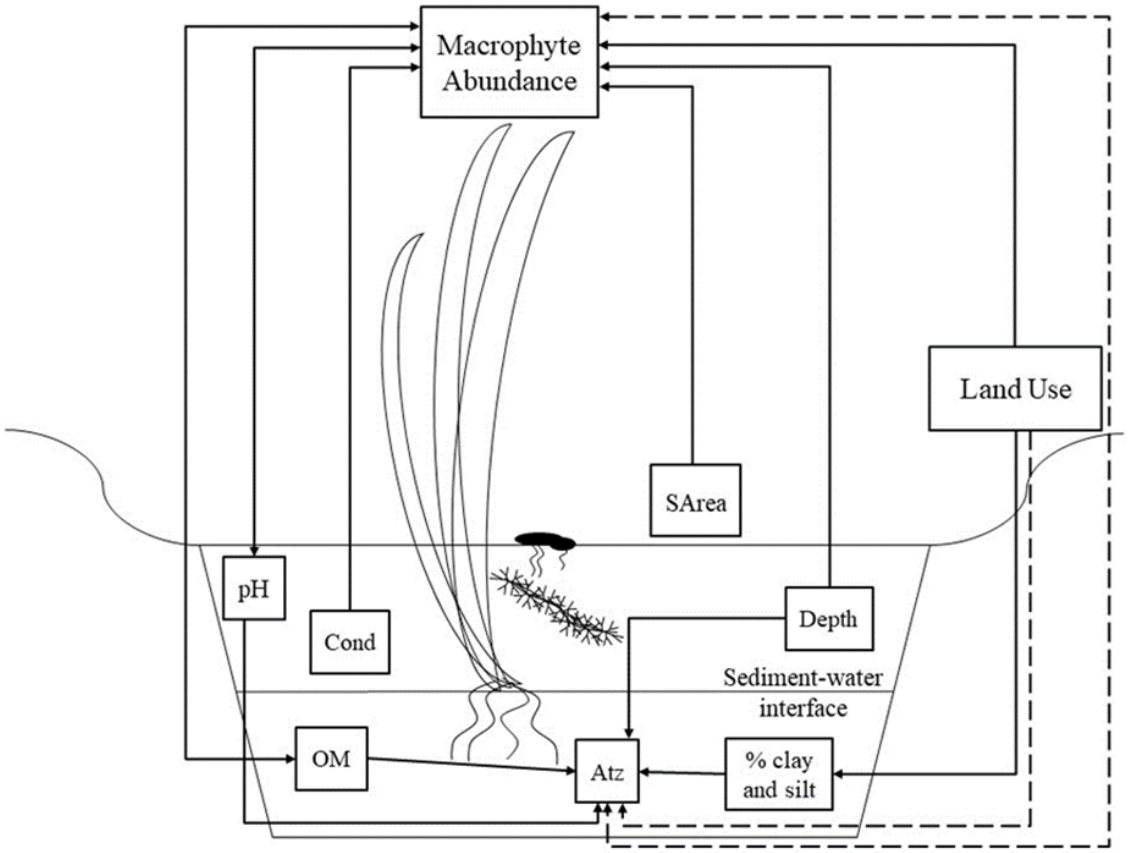

Figure 2. Conceptual diagram of known and hypothesized relationships between physical, biological, and chemical variables measured in this study (macrophyte abundance: composition and frequency of emergent, floating, and submerged species; land use: agricultural, conservation, golf course; water variables: pH, Cond = conductivity, SArea = open water area, depth; sediment variables: OM = organic matter content, Atz = atrazine concentrations, clay and silt). Solid lines represent variables found to have relationships in the literature, dashed lines represent variables hypothesized to have relationships in this study.

MATERIALS AND METHODS

Study Site Descriptions

Twenty ponds were sampled in June and July of 2017 (Appendix I). These included ten ponds in the vicinity (within 0 – 10 m) of agricultural activity, five within a managed conservation area, and five in the vicinity (within 2 – 20 m) of golf courses. Sampling sites were located in Barton, Christian, Dade, Dallas, Greene, Hickory, Lawrence, Newton, and St. Clair counties in southwest Missouri, in the Ozarks region (Figure 3). Naturally formed ponds found in the Ozarks region are a result of Missouri's karst topography; however, most ponds in the region have been constructed and are used for aesthetics, farming, wildlife habitat, and recreation (MDC 2018).

Ponds in the vicinity of agriculture appeared to be the most different in terms of open water area (38 ha – 14,139 ha), depth (< 0.10 m – 3 m), and surrounding activity. Surrounding agricultural land included various crops (alfalfa, corn, fescue grass, sorghum, soybean, and wheat) and cattle farming. All conservation ponds (open water area: 4 ha – 253 ha; depth: 0.10 m – 0.45 m) were located within the Drury-Mincy Conservation Area, which is heavily forested, but managed (e.g., wildlife food plots, prescribed fire). This area has no known atrazine application, however glyphosate (Roundup®), fluroxypyr ester, and triclopyr ester (Pasture Guard® and Remedy®) are lightly applied through spray and foliar applications along roadsides only (Nick Shortt, email communication 2017). Golf course ponds (open water area: 45 ha – 1097 ha; depth: 0.10 m – 2.5 m) were in the vicinity of recreational activities such as golfing and hiking, or bike trails. Surrounding vegetation in these areas were consistently maintained through

fertilizer and herbicide applications, and mowing. Details regarding herbicide application (i.e., active ingredient, application technique, application rate, frequency of use) could not be determined for the agricultural and golf course ponds in this study.

Macrophyte Surveys

Macrophyte surveys were conducted in each pond to determine species cover (Appendix II), composition (Appendix III) and frequency (Appendix IV). Three equally spaced transects were established across the width of each pond. A PVC quadrat (0.75 x 0.75 m) was deployed at ten random locations along these transects (Figure 4). Macrophyte and algae species occurring within the quadrat were identified through a plexiglass-bottomed cylinder (when necessary), and their percent cover determined using the Daubenmire method (Daubenmire 1959). Species floating over or touching the quadrat frame were also included in the percent cover determinations. As a result, percent cover could exceed 100% due to all macrophyte groups being estimated (i.e., floating and submerged). Percent cover estimates were used to calculate macrophyte composition and frequency, using the following equations (Daubenmire 1959);

$$\text{Cover (\%)} = \left[\frac{\Sigma\ (\#\text{ of quadrats in cover class (per species)} \times \text{cover class midpoint})}{\text{total \# of quadrats}}\right]$$

$$\text{Frequency (\%)} = \frac{\#\text{ of occurrences of a species (\# of quadrats species was observed)}}{\text{total \# of quadrats}} \times 100$$

$$\text{Composition (\%)} = \frac{\text{cover (\%) of each species}}{\text{total cover of all species}}$$

Where:

Cover class: 1 = 1-5%; 2 = 5-25%; 3 = 26-50%; 4 = 51-75%; 5 = 76-95%; 6 = 96-100%

Cover class midpoint: 1 = 2.5%; 2 = 15%; 3 = 37.5%; 4 = 62.5%; 5 = 85%; 6 = 97.5%

Emergent species present along the shorelines of all transects were also identified.

Water and Sediment Collection and Analysis

A multi-parameter meter (VWR symphony H30PCO) was used to measure pH and conductivity of the surface water (~20 cm deep) at five random locations along the three transects. Water depth was estimated using a scale on the sides of a PVC corer apparatus (Figure 5). Open water area of each pond was determined by averaging triplicate area measurements estimated using Google Earth images in ImageJ, an image processing software. The PVC corer was also used to collect approximately 10 cm of surface sediment at the same five random locations where water pH and conductivity were measured (Figure 4). Sediment samples were placed in plastic bags and stored on ice for transport to the lab for processing and analysis.

A subsample of each sediment sample was used in a liquid-liquid atrazine extraction method adapted from Amadori et al. (2013). Approximately 8 g (± 0.05 g) of fresh sediment sample was placed in 50 mL polypropylene centrifuge tubes. Each sample was fortified with 0.3 mL of internal standard (Sigma Aldrich atrazine-d_5) for instrumentation comparison purposes only. A volume of 20 mL of extraction solution, composed of 80% Acetonitrile and 20% HPLC water, was added to each sediment sample. Samples were inverted to loosen the sediment and disperse the internal standard and extraction solution throughout the sample. After inverting, samples were mixed vigorously using a wrist-action shaker (Fisherbrand™) at approximately 400 rpm for one hour, and then centrifuged at 2500 rpm for ten minutes. Each sample supernatant was decanted into clean 50 mL polypropylene tubes. An additional 20 mL of extraction

solution was added to the sediment sample, and the extraction steps were repeated to yield 40 mL of final sample extract. Each final extract was filtered using a pressure filter holder with a 0.45 μm membrane filter. A standard reference curve with atrazine concentrations at 3.0 ng mL^{-1}, 1.5 ng mL^{-1}, 0.75 ng mL^{-1}, 0.25 ng mL^{-1}, and 0.1 ng mL^{-1} was prepared using atrazine analytical standard obtained from Sigma Aldrich (Supelco). Reference curve points were prepared using serial dilutions, starting with a 100 μg mL^{-1} stock standard. All standards were prepared with 80% Acetonitrile:20% HPLC water. All samples and standards were analyzed by LC-MS/MS equipped with an autoinjector, using a Phenomenex Kinetex XB-C18 column (50 mm x 2.1 mm, 1.7 μm), and turbo spray ion source. Aqueous mobile phases consisted of 0.1% formic acid in water (A), and 0.1% formic acid in methanol (B). Injection volume was 10 μL, and atrazine retention time was approximately 2.03 minutes. The instrument's lowest limit of detection (LOD), 0.03 ng mL^{-1}, was calculated as three times the standard deviation of the lowest reference curve point (Smith 1993). Atrazine concentrations of my samples were about 10 times higher than the instrument's LOD.

The remaining fresh sediment samples were transferred to paper bags and placed in a drying oven at 60 °C until reaching a constant dry weight (g). Each sediment sample was ground using ceramic mortar and pestle, homogenized, and passed through a 2 mm stainless steel sieve. Percent organic matter content (OM) was determined using a loss on ignition (LOI) method adapted from the North Central Regional Research Chemical Soil Test Procedures (Combs and Nathan 1998). Approximately 5 g of each sediment sample was dried at 105 °C for two hours in a muffle furnace (Thermo Scientific Thermolyne). Samples were removed from the oven, allowed to cool to room temperature, and their

weights measured. The samples were returned to the muffle furnace to ash at 360 °C for another 2 hours. The temperature was then lowered and the samples removed after the muffle furnace reached 150 °C. Samples were allowed to cool to room temperature, and weighed. Replicates of two *Agro*MAT standard reference materials (clay soil, AG-1 and sandy soil, AG-2) were included with each batch of sediment samples to determine average percent recovery. Average recovery percentages were 79% and 87% for AG-1 and AG-2. Percent organic matter content was calculated using the following equation;

$$\text{Organic matter content (\%)} = \left[\frac{(\text{weight at 105 ℃ (g)}) - (\text{weight at 360 ℃ (g)})}{\text{weight at 105 ℃ (g)}}\right] \times 100$$

Particle size was determined using methods adapted from the USGS Laboratory Theory and Methods for Sediment Analysis (Guy 1969). The ashed sediment samples were poured into a 63 μm sieve and rinsed thoroughly with tap water to wash away clay and silt, and retain the sand particles. The remaining sand particles were washed into pre-weighed Whatman grade-4 filter paper and allowed to drain. These filters were then carefully folded and placed in an oven at 60 °C for 48 hours, or until constant weight. The following equations were used to calculate the percent fraction less than 63 μm (that is, the percent clays and silts);

$$\text{Sand particle weight ((g))} = \text{dry weight (soil + filter)} - \text{filter weight}$$

$$\text{Clays and silts (\%)} = \left[\frac{(\text{ashed weight} - \text{sand particle weight})}{\text{dry weight}}\right] \times 100$$

Statistical Analysis

Prior to statistical analyses, water and sediment variables were transformed to fit data into normal distributions using the following transformations in Minitab 17:

sediment atrazine concentrations and sediment organic matter content – Box-Cox; conductivity, open water area, and particle size – Johnson; pH, and depth did not require transformation. Additionally, macrophyte variables (composition and frequency) were relativized by maxima and arcsine transformed to equalize variance (McCune and Grace 2002). Rare macrophytes ($n<2$) were removed in composition and frequency matrices (McCune and Grace 2002). One-way ANOVA and Tukey's multiple comparison tests were performed in Minitab 17 to determine significant differences between pond categories for water depth, open water area, pH, and conductivity; sediment atrazine concentrations, organic matter content, and particle size; and macrophyte composition, frequency, cover, and richness. Significance was determined using a Bonferroni correction ($\alpha=0.017$) to reduce the chances of type I errors (McCune and Grace 2002).

Indicator Species Analyses (ISA) was performed in PC-ORD v. 6.0 using a randomization test with quantitative or binary response data (Dufrêne and Legendre 1997) to determine significant indicator species ($p<0.05$) for the different pond categories using macrophyte composition and frequency data, and emergent macrophyte presence-absence data.

Detrended Correspondence Analyses (DCA), Canonical Correspondence Analyses (CCA), and Redundancy Analysis (RDA) were performed in Canoco5 using macrophyte (composition, frequency, and presence-absence) and environmental variables (water depth, open water area, pH, and conductivity; sediment atrazine concentrations, organic matter content, and particle size). Unconstrained DCA indicated that CCA was appropriate for analysis of macrophyte composition and frequency data because gradient lengths were greater than four; whereas a RDA was the appropriate analysis for

macrophyte presence-absence data because gradient lengths were less than four (Šmilauer and Lepš 2014). To determine relatedness between macrophyte composition and frequency, and environmental variables, constrained CCAs were performed; and a constrained RDA was performed to determine relationships between macrophyte presence-absence and environmental variables. Variance inflation factors (VIF) were less than five indicating that variance was not inflated due to strong correlations between environmental variables. Significant environmental variables to be included in final models were identified using forward selection (p<0.05) with Monte Carlo permutation tests (499 unrestricted permutations) (ter Braak and Šmilauer 2012). Variance attributed to these environmental variables were partitioned according to methods by Borcard et al. (1992) and Legendre (2007).

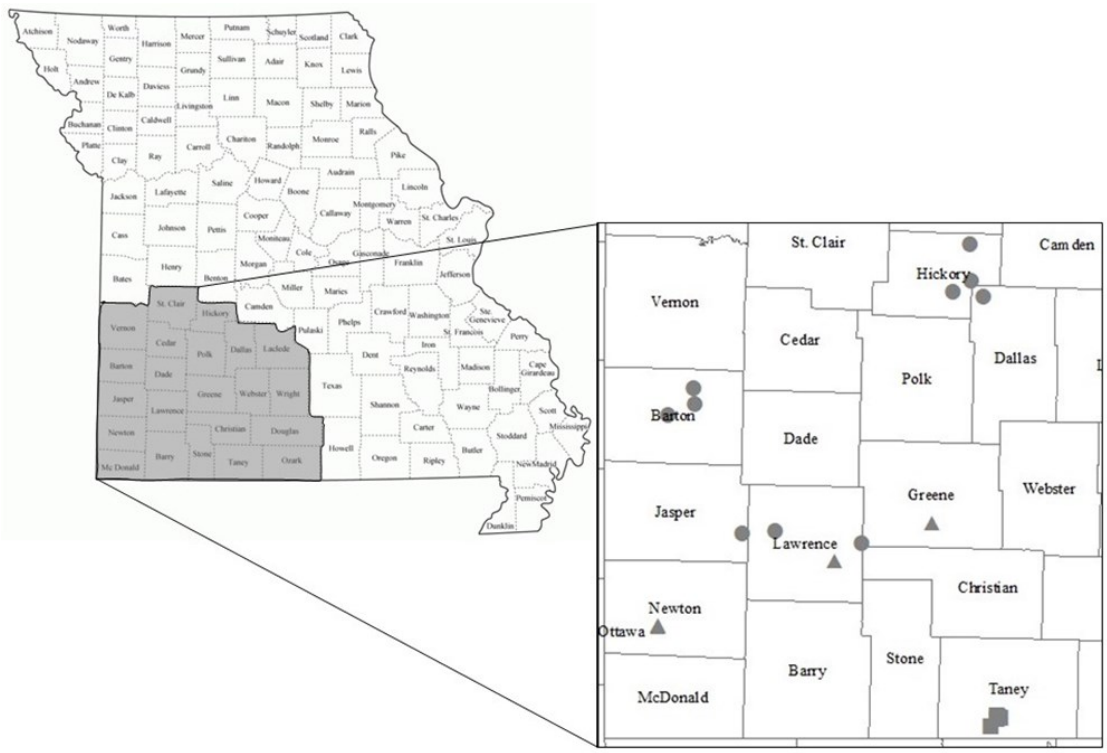

Figure 3. Map of Southwest Missouri counties with the locations of pond study sites (● agricultural (n=10), ■ conservation (n=5), ▲ golf course (n=5)).

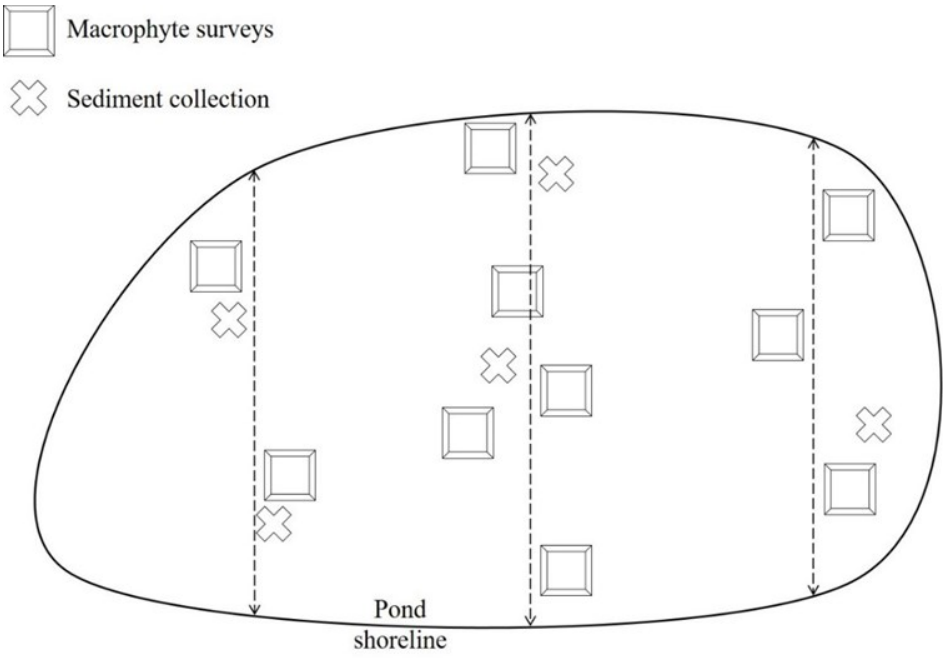

Figure 4. Hypothetical transect locations for a typical pond study site with macrophyte surveys representing quadrat locations and sediment collection representing sediment core and water analysis sample locations.

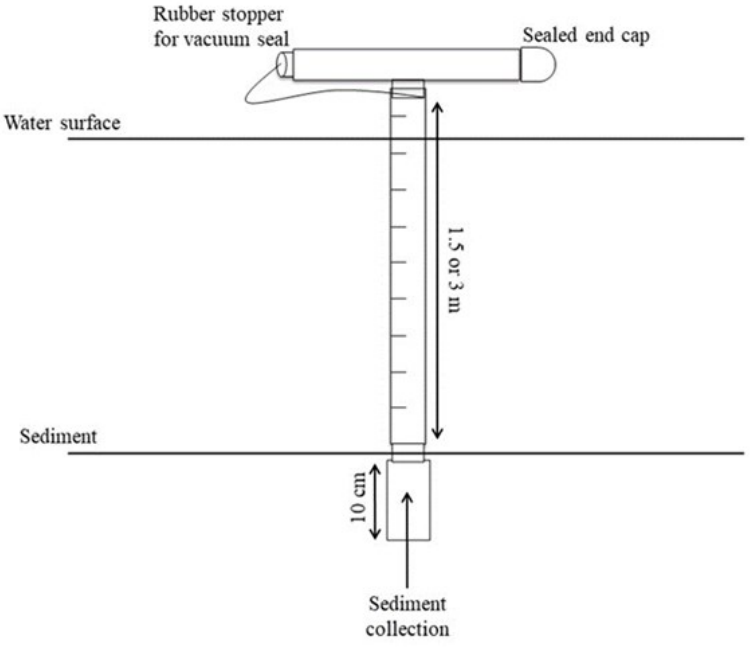

Figure 5. Diagram of PVC sediment corer used to collect sediment in each pond (modified from Madsen et al. 2007 and Kissoon et al. 2015).

RESULTS

Macrophytes: Differences Among Pond Categories

Macrophyte and algae communities (emergent, floating, and submerged species) varied between pond categories (Appendix V). Macrophyte and algae richness were similar in agricultural and golf course ponds, compared to conservation ponds (Figure 6). Macrophyte and algae cover was also similar among pond categories, however the trend was conservation > golf course > agricultural (Figure 7). Conservation ponds were consistently covered in free-floating macrophytes (Lemnaceae spp.). Indicator species analyses of macrophyte variables (composition and frequency) identified three macrophytes as indicator species in this study. *Lemna minor* and *Spirodela polyrhiza* were significant indicators for conservation ponds; whereas *Spirogyra* spp. was a significant indicator for golf course ponds (Tables 2 and 3). No indicator species were identified for emergent macrophytes. Emergent species most often observed on the shorelines of each pond category were as follows, agricultural ponds: *Carex frankii*, *Leersia oryzoides, Rumex crispis*, and *Salix nigra*; conservation ponds: *Elymus jejunes*, *Elymus elymoides*, *Hordeum jubatum*, and *Setaria viridis*; golf course ponds: *Acer saccharum*, *Apocynum* sp., *Eleocharis obtusa*, *Scirpus cyperinus*, *Typha latifolia*, and *Ulmus* sp.

Environmental Variables: Differences Among Pond Categories

Water pH and open water area were significantly higher ($p<0.001$) in agricultural ponds compared to conservation and golf course ponds (agricultural > golf course >

conservation), and water depth was significantly lower in conservation ponds (conservation < golf course < agricultural) (Figure 8). Water conductivity did not significantly differ among pond categories. Sediment organic matter content (OM) was significantly higher (p<0.001) in conservation ponds compared to agricultural and golf course ponds (Figure 9). Sediment atrazine concentrations and particle size (% clay and silt) did not significantly differ among pond categories.

Relationships Among Macrophyte and Environmental Variables

Results of the CCAs indicated that sediment OM and water pH were significant predictors of macrophyte and algae composition and frequency, explaining 27% of the total variation. OM was the most important predictor; whereas pH explained the least variation (Table 4). *L. minor, Persicaria punctata*, and *S. polyrhiza* were most associated with high OM and conservation ponds (Figure 10). *Cladophora* spp. and *Nelumbo lutea* were most associated with high water pH and agricultural ponds; whereas *Ludwigia palustris, Ludwigia peploides, Potamogeton foliosus, Spirogyra* spp., and *Zannichellia palustris* were most associated with golf course ponds (Figure 10).

Results of the RDA indicated that water depth and open water area were significant predictors of macrophyte and algae presence, explaining 24.5% of the total variation. Water depth was the most important predictor; whereas open water area explained the least variation (Table 4). *Spirogyra* spp. and *Apocynum* sp. were most associated with lower water depth; whereas *Cladophora* spp. was most associated with larger open water area and agricultural ponds (Figure 11).

Table 2. Indicator values and associated *p*-values for macrophyte and algae species based on composition data for agricultural (*n*=8), conservation (*n*=4), and golf course (*n*=4) ponds as determined by indicator species analysis (*p*-values indicate the probability of the listed indicator values given the species distributions, ns denotes no significance at α=0.05).

Pond category maximum group	**Macrophyte**	**Indicator value**	***p*-value**
Agricultural	*Cladophora* spp.	45.7	ns
	Nelumbo lutea	25.0	ns
	Zannichellia palustris	37.5	ns
Conservation	*Ceratophyllum demersum*	26.4	ns
	Lemna minor	60.5	0.04
	Persicaria punctata	50.0	ns
	Spirodela polyrhiza	74.0	0.01
	Wolffia brasiliensis	8.9	ns
	Wolffia sp.	48.8	ns
Golf Course	*Ludwigia palustris*	16.4	ns
	Ludwigia peploides	23.7	ns
	Potamogeton foliosus	23.3	ns
	Spirogyra spp.	71.5	0.01

Table 3. Indicator values and associated *p*-values for macrophyte and algae species based on frequency data for agricultural (*n*=8), conservation (*n*=4), and golf course (*n*=4) ponds as determined by indicator species analysis (*p*-values indicate the probability of the listed indicator values given the species distributions, ns denotes no significance at α=0.05).

Pond category maximum group	**Macrophyte**	**Indicator value**	***p*-value**
Agricultural	*Cladophora* spp.	34.5	ns
	Nelumbo lutea	25.0	ns
	Zannichellia palustris	37.5	ns
Conservation	*Ceratophyllum demersum*	24.7	ns
	Lemna minor	67.0	0.01
	Persicaria punctata	50.0	ns
	Spirodela polyrhiza	66.3	0.01
	Wolffia sp.	46.2	ns
	Wolffia brasiliensis	14.2	ns
Golf course	*Ludwigia palustris*	22.0	ns
	Ludwigia peploides	43.0	ns
	Potamogeton foliosus	18.8	ns
	Spirogyra spp.	68.6	0.009

Table 4. Results of constrained analyses models for macrophyte and algae composition, frequency, and presence-absence using environmental variables as explanatory variables (determined by forward selection with Monte Carlo permutation tests (499 unrestricted permutations) (ter Braak and Šmilauer 2012), $p<0.05$).

Macrophyte matrix	Environmental variable	Explained variance (%)	*p*-value	Model
Composition	Sediment organic matter content	15.1	0.006	CCA
	Water pH	11.7	0.022	
	Total	26.8		
Frequency	Sediment organic matter content	15.2	0.008	CCA
	Water pH	12.1	0.022	
	Total	27.3		
Presence-absence	Water depth	15.0	0.002	RDA
	Open water area	9.5	0.01	
	Total	24.5		

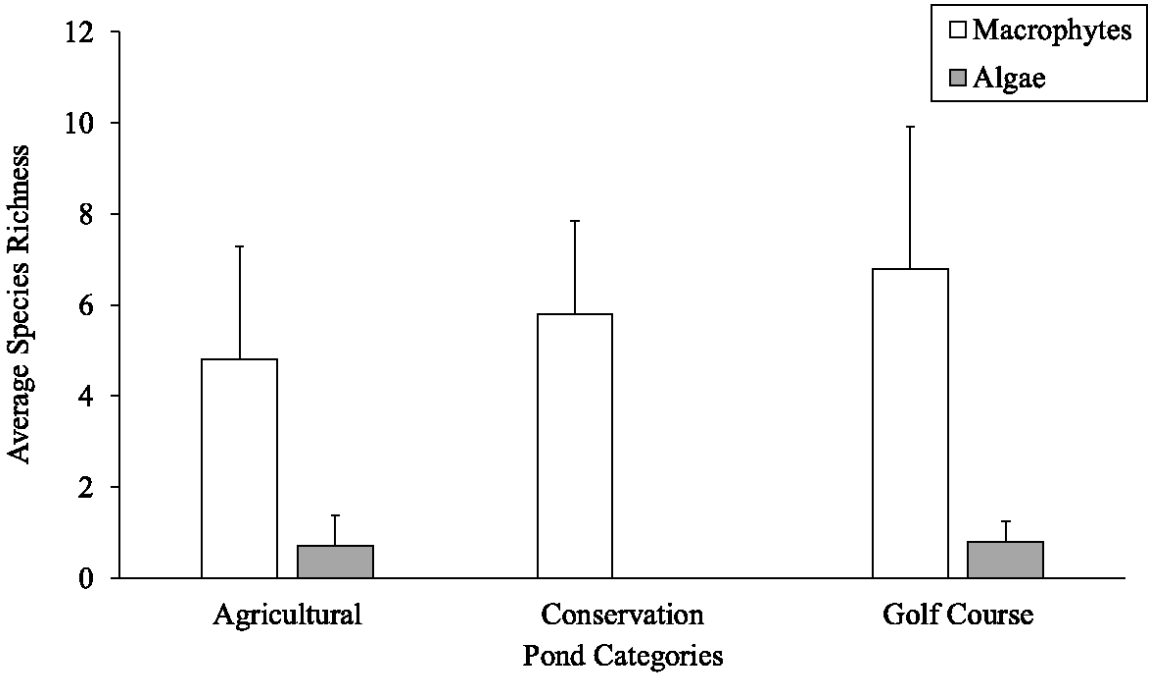

Figure 6. Average macrophyte and algae richness for agricultural (*n=10*), conservation (*n=5*), and golf course (*n=5*) ponds (error bars represent standard deviation).

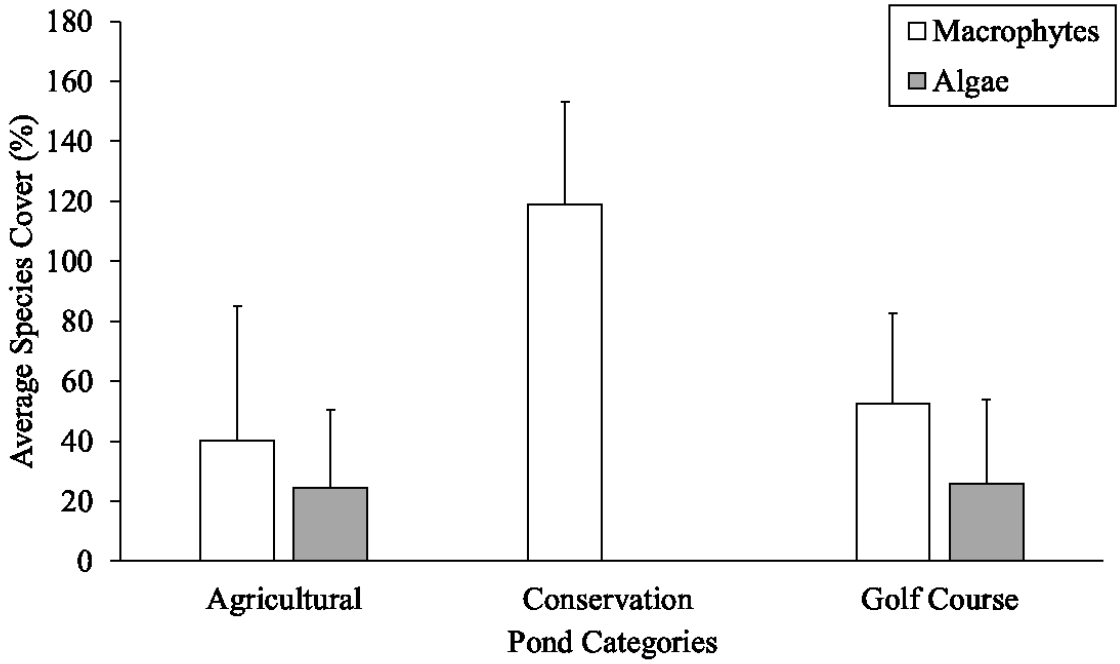

Figure 7. Average macrophyte and algae cover for agricultural (*n=10*), conservation (*n=5*), and golf course (*n=5*) ponds (error bars represent standard deviation).

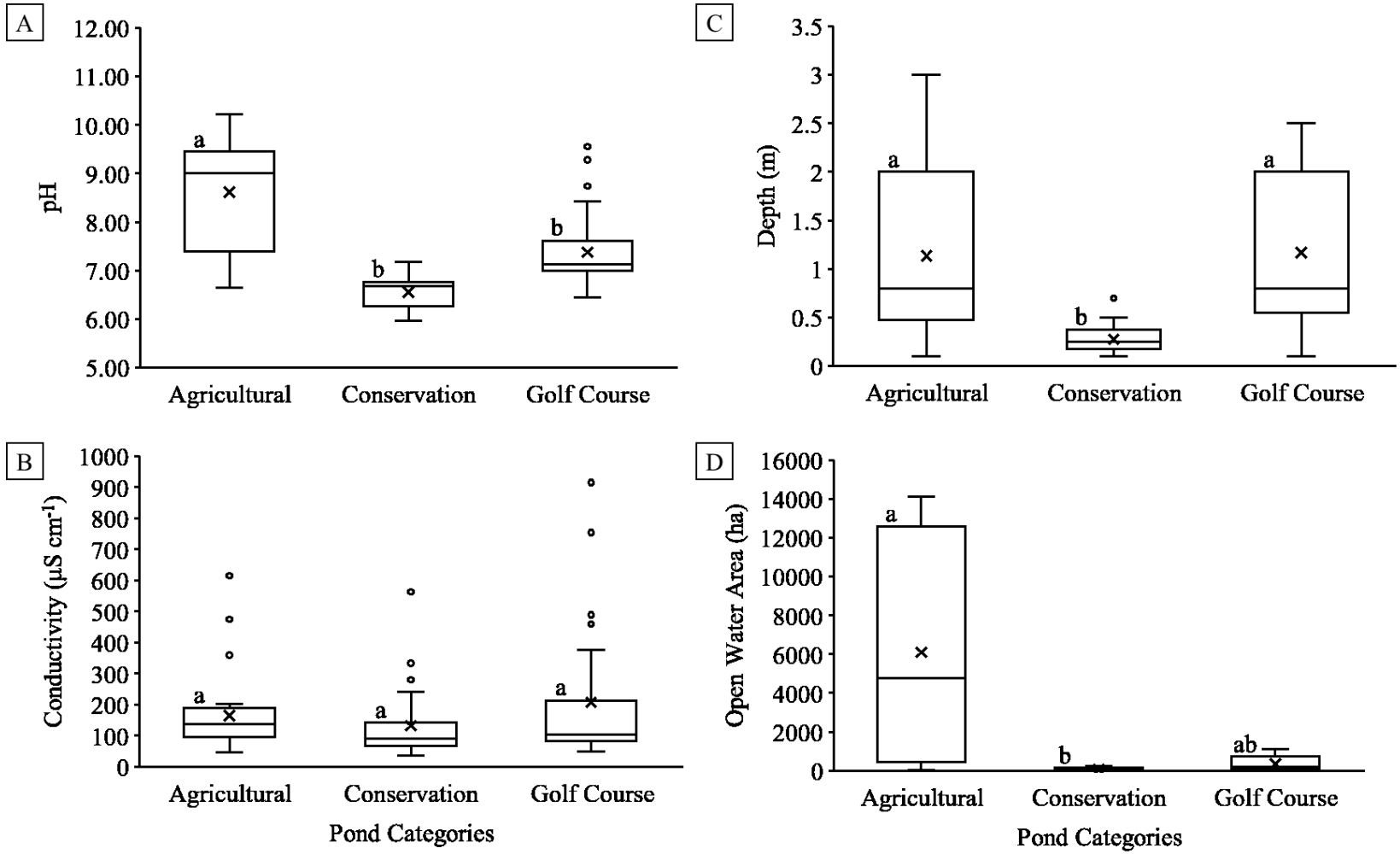

Figure 8. Boxplots comparing pond categories (agricultural (n=10), conservation (n=5), and golf course (n=5)) for four water variables; (A) pH, (B) conductivity (µS cm^{-1}), (C) depth (m), (D) open water area (ha) (different letters indicate significant differences, p<0.001).

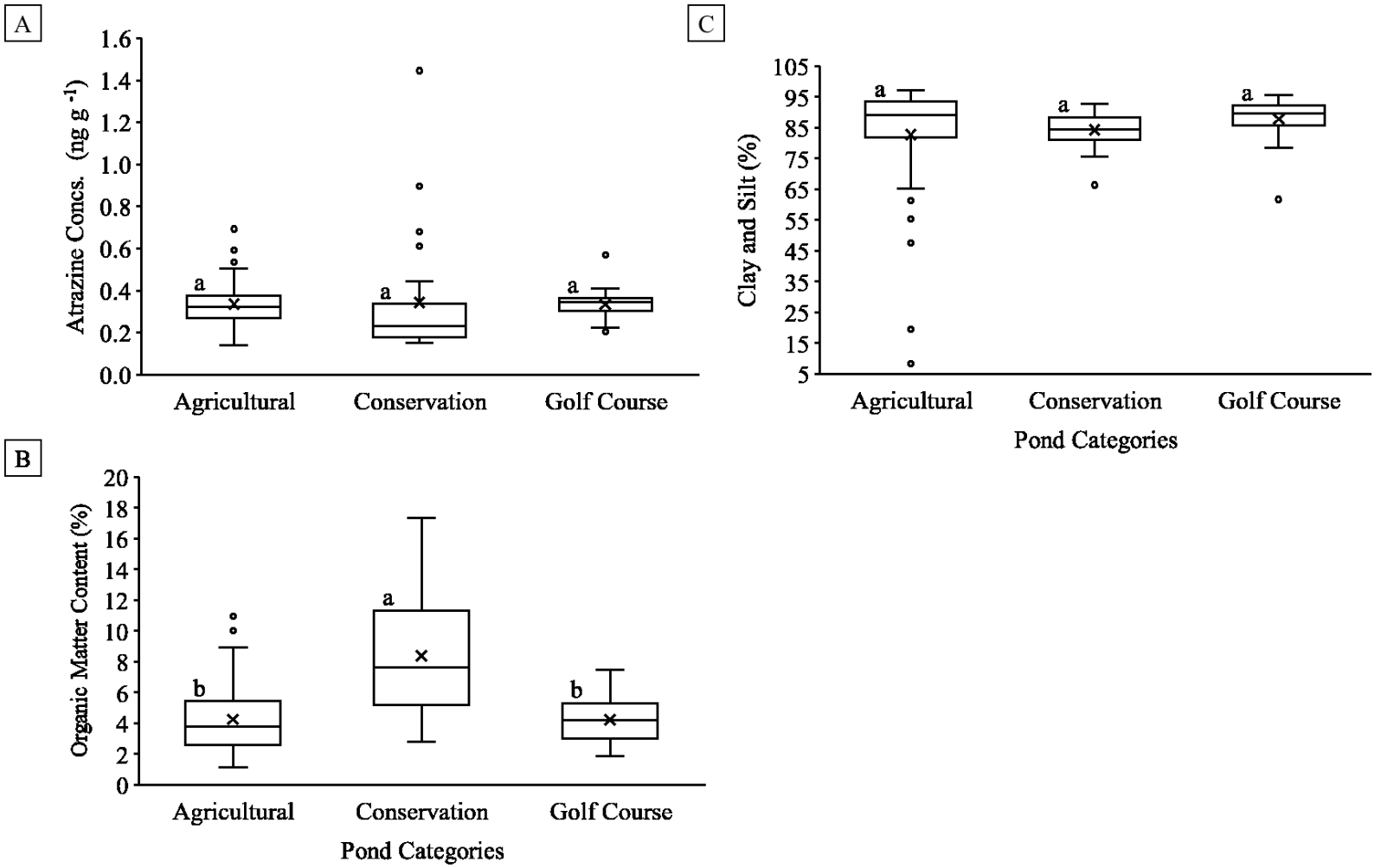

Figure 9. Boxplots comparing pond categories (agricultural (n=10), conservation (n=5), and golf course (n=5)) for three sediment variables; (A) atrazine concentrations (ng g^{-1}), (B) organic matter content (%), (C) clay and silt (%) (different letters indicate significant differences, p<0.001).

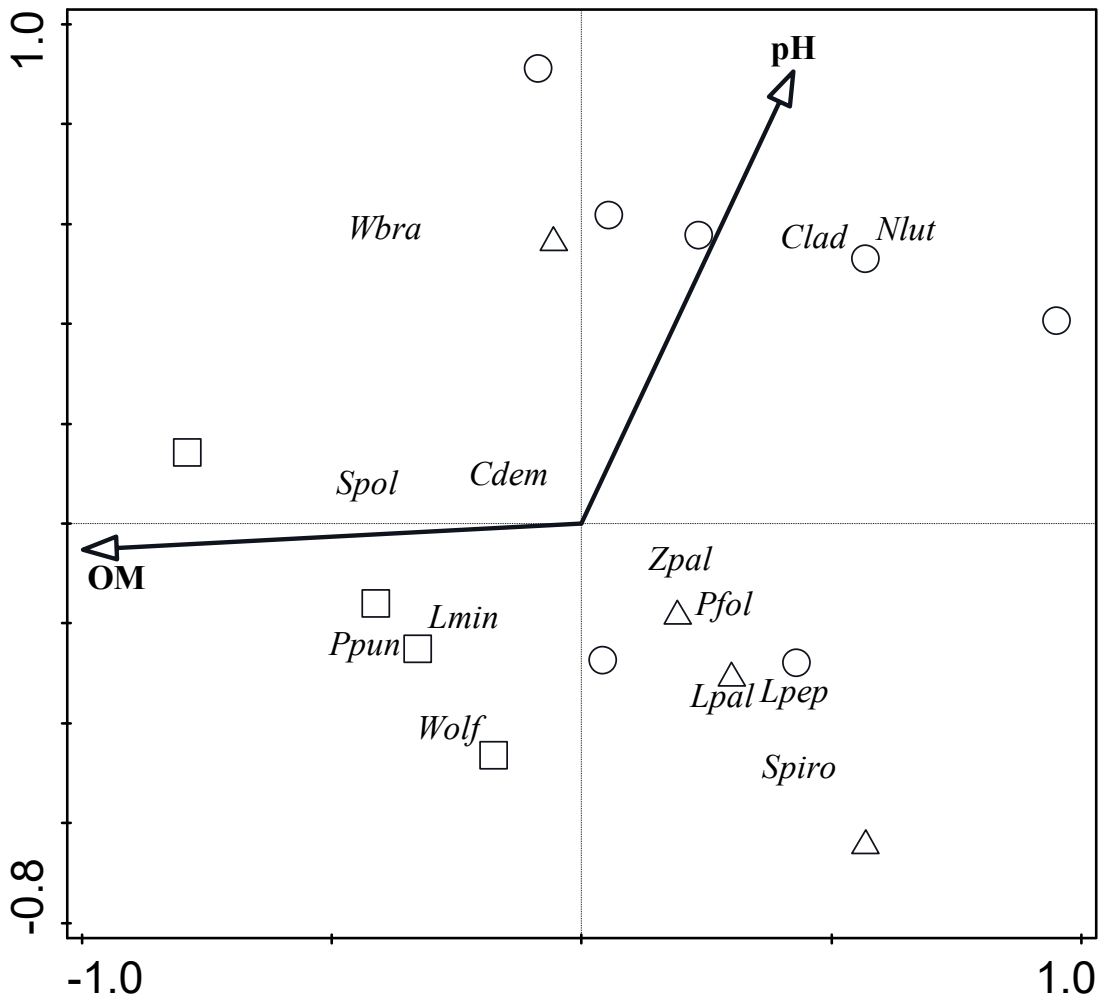

Figure 10. Ordination triplot of the CCA showing macrophyte and algae composition of the different ponds constrained by significant environmental variables (ponds: ○ = agricultural (*n*=7), □ = conservation (*n*=4), Δ = golf course (*n*=4); environmental variables (bold): pH = water pH and OM = sediment organic matter content; macrophytes: *Cdem = Ceratophyllum demersum, Clad = Cladophora* spp., *Lmin = Lemna minor, Lpal = Ludwigia palustris, Lpep = Ludwigia peploides, Nlut = Nelumbo lutea, Pfol = Potamogeton foliosus, Ppun = Persicaria punctata, Spiro = Spirogyra* spp., *Spol = Spirodela polyrhiza, Wbra = Wolffia brasiliensis, Wolf = Wolffia* sp., *Zpal = Zannichellia palustris*).

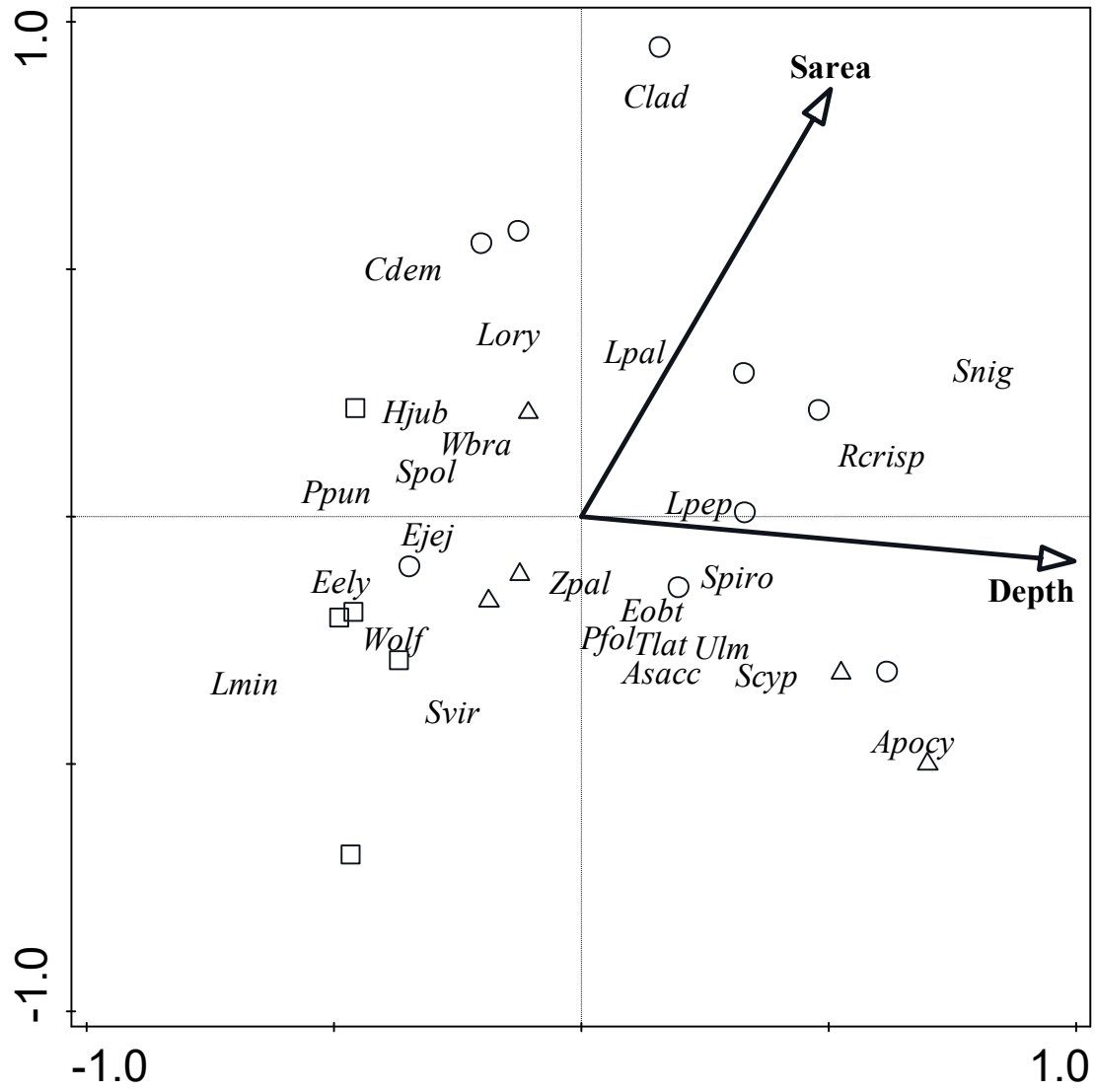

Figure 11. Ordination triplot of the RDA showing macrophyte and algae presence-absence for the different ponds constrained by significant environmental variables (ponds: ○ = agricultural (*n*=9), □ = conservation (*n*=5), Δ = golf course (*n*=5); environmental variables (bold): Sarea = open water area and Depth = water depth; macrophytes: *Apocy = Apocynum* sp., *Asacc = Acer saccharum, Cdem = Ceratophyllum demersum, Clad = Cladophora* sp., *Eely = Elymus elymoides, Ejej = Elymus jejunes, Eobt = Eleocharis obtusa, Hjub = Horbeum jubatum, Lmin = Lemna minor, Lory = Leersia oryzoides, Lpal = Ludwigia palustris, Lpep = Ludwigia peploides, Pfol = Potamogeton foliosus, Ppun = Persicaria punctata, Rcrisp = Rumex crispus, Scyp = Scirpus cyperinus, Snig = Salix nigra, Svir = Seratia viridis, Spiro = Spirogyra* spp., *Spol = Spirodela polyrhiza, Tlat = Typha latifolia, Ulm = Ulmus* sp., *Wbra = Wolffia brasiliensis, Wolf = Wolffia* sp., *Zpal = Zannichellia palustris*).

DISCUSSION

Results of the current study demonstrated that multiple environmental characteristics can impact macrophyte communities in ponds (Figure 12), whereas previous literature tended to focus on the effects of either water or sediment characteristics, or only single environmental variables. Unlike previous work, the current study considered relationships between macrophytes, and various water and sediment characteristics, including water pH, conductivity, depth, open water area, sediment organic matter content, particle size, and atrazine concentrations in ponds in the vicinity of agricultural, conservation, and golf course land use. Findings of this work were, (1) macrophyte communities and water and sediment characteristics varied among ponds; (2) sediment atrazine concentrations were similar among ponds; and (3) multiple environmental characteristics played a role in macrophyte composition, frequency, and presence-absence.

Wetland sediments reflect past events and disturbances, and represent longer time periods compared to water analyses, which represent shorter time periods or single events (Håkanson and Jansson 1983; Werkmeister et al. 2018). As such, this should be a preferred variable when measuring pollutants in wetlands. Sediment composition and chemistry play an important role in plant growth (Barko et al. 1991; Dong et al. 2017; Verhofstad et al. 2017), yet few studies have examined relationships between pond sediment characteristics and macrophyte communities (Lougheed et al. 2001; Kissoon et al. 2013). My study focused on both pond water and sediment characteristics to determine possible impacts on macrophyte communities.

Macrophytes: Differences Among Pond Categories

Macrophytes varied between pond categories, and ordination plots showed that ponds grouped together according to land use categories, indicating a relationship between macrophyte communities and surrounding land use. Similarly, several studies reported that macrophyte abundance, richness, and presence were influenced by surrounding land use (Povidisa et al. 2009; del Pozo et al. 2011; Mikulyuk et al. 2011). In this study, *Lemna minor* and *Spirodela polyrhiza* were indicator species of conservation ponds. These ponds were characteristically suitable for duckweed species, as they prefer undisturbed or stagnant waters (Hillman 1961), which could explain their dominant occurrence in the conservation ponds, which were quiet and sheltered from wind by surrounding trees. However, nutrient input is usually the driving factor for Lemnaceae spp. occurrence (Portielje and Roijackers 1995; Scheffer et al. 2003). Nutrient-rich ponds consistently contain dense mats of Lemnaceae spp. because of its tolerance to high nutrient concentrations (Lougheed et al. 2008; Povidisa et al. 2009). Additionally, many macrophytes found in this study were also reported to be tolerant or moderately tolerant of high nutrient conditions, including, *Carex* sp.*, Ceratophyllum demersum, Fraxinus* sp.*, Hordeum jubatum, Juglans nigra, Leersia oryzoides, Lemna minor, Myriophyllum spicatum, Nelumbo lutea, Phalaris arundinacea, Rumex crispus, Salix* sp.*, Scirpus cyperinus, Typha* sp.*,* and *Ulmus* sp. (EPA 2018). In this study, these species most often occurred in agricultural and golf course ponds, which could be and indicator of the nutrient status of these ponds.

Spirogyra spp., which also thrives in nutrient-rich conditions (Hainz et al. 2009), was an indicator species of golf course ponds in this study. Golf course management

practices commonly include the use of various pesticides and fertilizers (Guzmán and Fernández 2014). Golf courses in the Midwestern United States receive approximately 41 kg of nitrogen and 4 kg of phosphorus per hectare, per year (Kohler et al. 2004). Due to the regular and frequent use of these chemicals, excess amounts of nitrates and phosphates could be entering nearby surface waters and ponds through runoff (Cohen et al. 1999; Kunimatsu et al. 1999; Reicher et al. 2005). Excessive algal growth in aquatic ecosystems is a common indicator of eutrophication, which is caused by the input of excess nitrates and phosphates (Randall and Mulla 2001). Eutrophication, as a byproduct of over enrichment could explain the significant *Spirogyra* spp. occurrence found in the golf course ponds in this study. Agricultural ponds in this study had no significant indicator species, and had the lowest average species richness. Declines in submerged macrophyte richness is usually observed in areas of heavy agricultural impact (Rasmussen and Anderson 2005), probably due to decreased light availability or species competition caused by increased algal growth (Dong et al. 2015; Vestergaard and Sand-Jensen 2000). However, macrophyte richness has also been found to positively correlate with wetland surface area (Houlahan et al. 2006), and negatively correlate with water depth (Akasaka et al. 2010). Water depth was greatest in agricultural ponds in the current study, which could explain why they had the lowest average species richness, as macrophyte growth, specifically submerged species, are dependent on light availability, thus affected by water depth.

Water and Sediment Characteristics: Differences Among Pond Categories

Previous studies showed that local geology and land use within watersheds play significant roles in wetland water and sediment characteristics (Dauer et al. 2000; Tsai et al. 2007; Kissoon et al. 2015; Yan et al. 2016). These wetland characteristics can subsequently impact the distribution and abundance of macrophytes (Jackson and Charles 1988; Grosshans and Kenkel 1997; Grillas 1990; Heegaard et al. 2001; Mäkela et al. 2004; Akasaka et al. 2010; del Pozo et al. 2014; O'Hare et al. 2012). The current study found that ponds in the vicinity of different land use activities were significantly different in water pH, water depth, open water area, and sediment organic matter content.

Differences in water pH can be influenced by geology (Sliva and Williams 2001), activities within the watershed (Tong and Chen 2002), as well as the type and abundance of vegetation and microorganisms that are present (Raich and Schlesinger 1992). Water pH in southwest Missouri (Ozarks region) is typically a reflection of the geology of the region and ranges from 7.67 to 7.93 (Ozark PWS 2017). The pH of sites in this study ranged from 5.97 (conservation pond) to 10.22 (agricultural pond). In agricultural areas, runoff could contain excess nutrients such as nitrates and phosphates from pesticides and fertilizers (Tong and Chen 2002). The use of these nitrogen or phosphate-based fertilizers can either increase or decrease water and soil pH depending on the main active ingredient of the fertilizer. For example, Pierre (1928) found various fertilizer compounds caused an increase of H+ ions (ammonium sulfate > ammonium phosphate > saltpeter > ammonium nitrate > urea), resulting in decreased soil pH. On the contrary, agricultural land use has been found to positively correlate with pH (Renberg et al. 1993; Johnson et al. 1997). For example, Johnson et al. (1997) found that high alkalinity was associated with row crop

agriculture, and it was significantly explained by surrounding land use and geology within the ecotone. Additionally, submerged macrophytes can also affect pH during the uptake or release of carbon dioxide during photosynthesis and cellular respiration (Raich and Schlesinger 1992).

Differences in sediment OM among the different pond categories could be related to pond productivity and surrounding land use activities. OM content is directly related to the amount of organic carbon present in the ecosystem (Jenkinson et al. 1992). Through organism respiration and decomposition processes, carbon is continuously added to sediments (Mitsch and Gosselink 2015). Ponds with high productivity, and thus higher vegetation abundance typically result in higher OM due to the organic inputs after vegetation senescence at the end of each growing season (Cronk and Fennessy 2001). According to Meyers and Teranes (2001) macrophytes constitute the largest input of organic matter into sediments. Conservation ponds in this study were surrounded by heavy tree cover and were mostly covered with floating macrophytes, which likely explains the high OM found in these ponds. Whereas, agricultural and golf course ponds contained lower amounts of OM due to less vegetation occurring in these ponds and on the surrounding land.

Water depth differences between pond categories could be due to natural or anthropogenic construction, macrophyte presence, or surrounding land use, which has been found as an influential factor in previous research. Voldseth et al. (2007) found that wetland depth was highest when the surrounding land was managed, compared to unmanaged, where depth was lowest. Tsai et al. (2007) found that land use, as well as percent macrophyte cover influenced water depth due to higher evapotranspiration rates.

These studies support the water depth results in the present study, as the conservation ponds were constructed to be shallow for wildlife habitat within the conservation area, were left unmanaged after initial construction, were surrounded by heavily forested land, and had the lowest water depth. Open water area also significantly differed among pond categories. On average, open water area was greater in agricultural ponds, which corresponds with Dodson et al. (2005) where percent open water area was positively correlated with percent riparian agricultural land use. Additionally, open water area habitat can vary in wetlands and have an impact on macrophyte abundance (Kissoon et al. 2013).

Conductivity did not vary significantly among pond categories. However, on average the trend in conductivity was golf course < agricultural < conservation ponds. Like water pH, conductivity could also be impacted by fertilizer use due to the input of excess nutrients such as inorganic dissolved solids, including, chloride, nitrate, sulfate, and phosphate (EPA 2012).

Atrazine: Similarities Among Pond Categories

Atrazine concentrations in this study did not differ significantly among pond categories. However, these concentrations were comparable to atrazine concentrations detected in surface sediments collected from the Great Lakes (0.01 – 1.7 ng g^{-1}) (Guo et al. 2016). Results in the current study could be due to atrazine's extensive use, persistence, transport, and fate in the environment. Previous studies reported that atrazine concentrations varied in wetland sediments depending on multiple factors, including, soil pH (Jenks et al. 1998), water depth (Blume et al. 2004), sediment composition (Spark and

Swift 2002), organic matter content (Dunigan and McIntosh 1971; Zhu et al. 2018), redox status (Kruger et al. 1993; Seybold et al. 2001), vegetation presence (Rupassara et al. 2002), microbe-macrophyte interactions (Larson et al. 2004; Wang et al. 2012), and inundated vs. unsaturated conditions (Kruger et al. 1993; 1997). In the current study, water depth and sediment organic matter content varied among ponds categories, but macrophyte cover did not. The inundated conditions and presence of macrophytes in the ponds in this study could explain the lack of variation of sediment atrazine concentrations. In addition, all of the ponds sampled in this study were located within the Missouri and Arkansas-White River watershed, which accommodates crop farming, cattle farming, tourism, and golfing, which might be contributing to the ubiquitous atrazine concentrations detected in pond sediments in this study.

Atrazine's environmental fate is dependent on the availability and capability of abiotic and biotic degradation (Singh and Jauhari 2017). It is typically stable in the environment at 25 °C, within pH ranges of 5-9 (Solomon et al. 1996). The average pH values in this study were within that range at 6.6-8.6, which would enable stable atrazine concentrations within most of the ponds sampled. However, under acidic conditions, atrazine can be hydrolyzed, compromising its structure and potentially its toxicity (Franzen and Zollinger 1997; Weaver et al. 2004). Several studies have found that atrazine can also be removed or degraded in systems more effectively and more rapidly when macrophytes are present (Kruger et al. 1997; Moore et al. 2000, 2013, 2017; Runes et al. 2001; Rupassara et al. 2002; Blume et al. 2004; Guimarães et al. 2011; Wang et al. 2012). In controlled hydroponic experiments, *Ceratopyllum demersum, Elodea canadensis,* and *Lemna minor* were found to efficiently remove atrazine from water (Rice

et al. 1997). *C. demersum* and *L. minor* were present in 70% of ponds in this study and could possibly be contributing to the degradation of atrazine in these pond sediments.

In addition to the presence of macrophytes, the presence of inundated or saturated conditions might also play a role in atrazine mobility. Surface sediment collected from wetlands enrolled in USDA conservation programs, surrounded by croplands or grasslands in Colorado, Kansas, Nebraska, New Mexico, Oklahoma, and Texas, had about 16 times greater atrazine concentrations than ponds in the present study (Belden et al. 2012). Cropland wetlands had an average of 10.7 ng g^{-1} and grassland wetlands had an average of 2.8 ng g^{-1} atrazine. These wetlands differed from those in the current study in ecoregion, surrounding cropland species (e.g., cotton as the main crop), and were unsaturated at the time of sediment sampling. These differences might explain the differences in sediment atrazine concentrations between the two studies, as species presence and inundation appear to play key roles in atrazine persistence in sediment.

Sediment clay and OM also play key roles in atrazine adsorption (Jenks et al. 1998; Vonberg et al. 2014) thus affecting its persistence and mobility in sediments (Gao et al. 1998; Spark and Swift 2002). Previous studies reported that sediments with high OM and low clay content inhibited atrazine mobility due to higher adsorption rates between atrazine and OM particles (Jenks et al. 1998; Ling et al. 2006; Zhu et al. 2018). Dunigan and McIntosh (1971) found that only 40 μg g^{-1} of atrazine adsorbed to clay without OM, compared to 77.5 μg g^{-1} of atrazine that adsorbed to clay with OM. The clay and silt content of ponds in the current study were similar with an overall average of 85%, which might explain the similar concentrations of atrazine detected among pond categories. Additionally, atrazine concentrations did not differ with OM, however the

highest concentrations detected occurred in conservation ponds, which on average had significantly higher OM. While these results are contrary to typical interactions seen between atrazine and OM, this could be explained by comparing high sediment OM in this study to characteristically high sediment OM. Sediments determined as rich in OM, commonly have OM greater than 30% (Mitsch and Gosselink 2015), whereas OM in this study did not exceed 20%. Therefore, atrazine could have adsorbed to the OM present in these conservation ponds, however OM may not have been high enough to significantly decrease atrazine's bioavailability within these ponds. These higher concentrations might also be explained by the proximity of the conservation area to urbanized areas and heavy golf activity in the Arkansas-White river watershed.

Relationships Among Macrophyte and Environmental Variables

In the current study, macrophyte composition and frequency were found to be related to water pH and sediment organic matter content; whereas macrophyte presence-absence was found to be related to water depth and open water area. Differences among pond categories indicated that land use may also play a role in macrophyte distribution. Several studies have reported relationships among macrophyte communities and various water and sediment variables (Heegaard et al. 2001; Vestergaard and Sand-Jensen 2000; Lougheed et al. 2001; Mackie et al. 2004; Akasaka and Takamura 2011; Kissoon et al. 2013). Various previous studies have also reported relationships between macrophytes and land use (Heegaard et al. 2001; Lougheed et al. 2001; Houlahan et al. 2006; Floyd et al. 2009; Akasaka et al. 2010; del Pozo et al. 2011; Mikulyuk et al. 2011; Kissoon et al. 2013; Evans et al. 2014). These previous studies emphasized that water and sediment

characteristics play a role in the composition, frequency, and richness of macrophyte communities in wetlands.

Water chemistry has long been known to be an influencing factor of macrophyte growth and distribution (Bini et al. 1999; Steffen et al. 2014; Sleith et al. 2018). Water pH was the second most important environmental variable explaining variation in macrophyte composition and frequency in the current study. Vestergaard and Sand-Jensen (2000) studied macrophyte richness in regard to water pH and open water area in small, shallow lakes. They found that pH was a significant determinant for species richness (low in acidic waters, high in alkaline waters) in mesotrophic wetlands, and that richness increased with surface area in non-turbid wetlands (Vestergaard and Sand-Jensen 2000). These wetland characteristics were the most important environmental variables explaining a considerable amount of variation in macrophyte composition and presence in the current study.

Hydrology and physicochemical characteristics of water in wetlands has also been found to strongly influence macrophyte communities. In this study, water depth and open water area were important factors influencing macrophyte presence. These variables have been found in previous research to influence macrophyte growth and productivity (Klopatek and Stearns 1978; Grillas 1990; Mäkela et al. 2004). Casanova and Brock (2000) reported that hydrology, water depth, and flooding duration were major predictors of macrophyte communities. Various other studies have also found these variables, as well as water pH, to be significant predictors of variation in macrophyte communities and abundance (Jackson and Charles 1988; Grillas 1990; Grosshans and Kenkel 1997; Akasaka 2010; del Pozo et al. 2011). Open water area of wetlands enables the

heterogeneity of macrophytes (emergent, floating, and submerged), which subsequently provides habitat for multiple aquatic organisms (Cedergreen et al. 2005). Specifically, submerged macrophytes are also heavily dependent on water depth, as it impacts light availability (Middleboe and Markager 1997).

The physicochemical characteristics of wetland sediments have also been found to influence macrophyte communities. Sediment characteristics such as composition and nutrient availability influence macrophyte productivity, composition, and diversity (Barko et al. 1991). Sediment organic matter content was the most important environmental variable, predicting more than half of the explained variation in macrophyte composition and frequency in the current study. Mackie (2004) found that sediment composed of organic and inorganic substrates, compared to strictly inorganic, correlated with greater macrophyte diversity. Kissoon et al. (2013) found that sediment organic matter content, in addition to other environmental variables, explained variation in macrophyte biomass. Sediment organic matter content provides nutrients to macrophytes, as well as microorganisms, which aids in biogeochemical processes.

Future Research

Previous research, as well as this study affirms the impact that multiple environmental variables have on macrophyte communities. Macrophytes play a crucial role in the environment, as they are the base of the food chain, as well as an important indicator of ecosystem health (Capers et al. 2010). With the continuous increasing use of chemicals, ecosystem characteristics should be regularly assessed, beyond routine water quality monitoring. Continuous human population growth parallels continuous

agricultural growth (Boserup 1965), which subsequently parallels continuous pesticide use. Specifically, atrazine, one of the most commonly used herbicides in the United States, known endocrine disruptor, and possible human carcinogen should be continually studied. Future research should include (1) phytoremediation studies with various macrophytes to determine effectiveness of atrazine removal and tolerance of macrophyte species; (2) greenhouse experiments with known atrazine concentrations to determine the effects of different environmental conditions on fate (i.e., macrophytes vs. no macrophytes, inundated vs. unsaturated soils); and (3) larger geographical areas and greater sample size to encompass a range of environmental conditions and atrazine concentrations. Future research in this discipline would aid in understanding the impact of different environmental conditions on the mobility and impact of herbicides in wetland ecosystems.

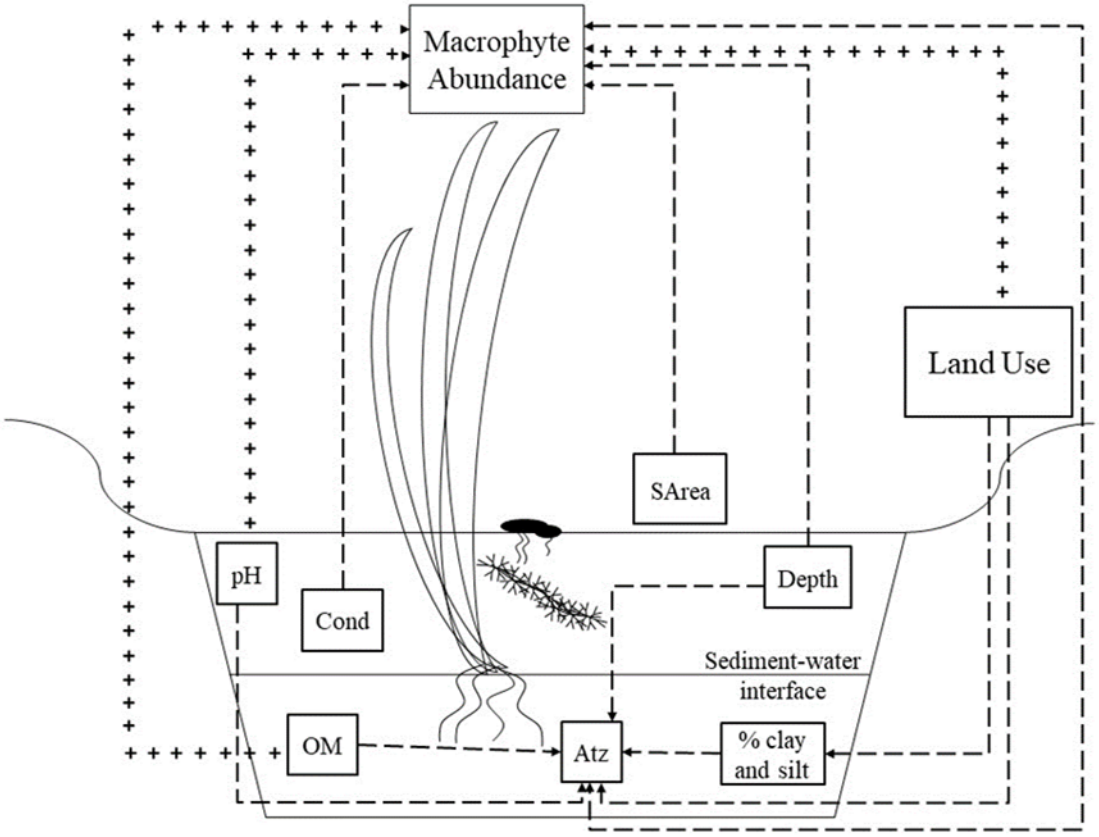

Figure 12. Conceptual diagram showing significant and non-significant relationships between physical, biological, and chemical variables measured in this study (macrophyte abundance: composition and frequency of emergent, floating, and submerged species; land use: agricultural, conservation, golf course; water variables: pH, Cond = conductivity, SArea = open water area, and depth; sediment variables: OM = organic matter content, Atz = atrazine, and percent clay and silt). Dashed lines represent variables found to have no relationship, and crossed lines represent variables found to have relationships.

CONCLUSION

This study found that land use, and water and sediment characteristics play important roles in macrophyte distribution and abundance in ponds. Water pH and sediment organic matter content were significant predictors of macrophyte composition and frequency, whereas water depth and open water area were significant predictors of macrophyte presence in ponds. Dominant land use also appeared to play a role in pond characteristics. High water pH and high open water area were associated with agricultural ponds, and high sediment organic matter content and low water depth were associated with conservation ponds. Specific macrophytes were found to be indicators of different pond categories. *Lemna minor* and *Spirodela polyrhiza* were identified as indicator species of conservation ponds, and *Spirogyra* spp. was identified as an indicator species of golf course ponds. Sediment atrazine concentrations were found to be similar in all pond categories, which could be due to its substantial and widespread use, persistence, and mobility in water and non-humic sediments. The findings of this study emphasize that pond and watershed characteristics play important roles in macrophyte communities, and thus should be collectively considered in the management of wetland ecosystems. Future work should include further phytoremediation and greenhouse studies, as well as field experiments on atrazine concentrations in areas with differing environmental conditions. Such research would broaden the knowledge on the relationships between macrophytes, atrazine, and water and sediment characteristics.

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APPENDICES

The following four appendices include raw data obtained at each agricultural, conservation, and golf course sampling site. Appendix I includes water and sediment data, as well as notes taken in the field. Appendix II includes macrophyte cover data, which was used to calculate macrophyte composition and frequency (data included in Appendix III and IV). Lastly, Appendix V shows macrophyte presence at each sampling site, as well as known atrazine resistance of each species, obtained from the literature.

Appendix I. Descriptive Information on Sampling Sites

Ia. Descriptive information for each agricultural pond including site number, location, average water (pH, Cond = conductivity (µS cm^{-1}), depth (m), and SArea = open water area (ha)) and sediment (Atz = atrazine concentrations (ng g^{-1}), OM = organic matter content (%), clay and silt (%)) variables, and field notes.

		Water variables				Sediment variables			
Site	**City**	**pH**	**Cond**	**Depth**	**SArea**	**Atz**	**OM**	**Clay and silt**	**Field Notes**
1	Lamar	9.01	178.0	0.54	8995	0.35	5.06	92.34	adjacent to wheat, 3 m of *Phalaris arundinaceae* buffer
2	La Russel	6.84	47.14	1.42	1646	0.29	5.37	77.48	high turbidity
3	La Russel	7.37	106.9	1.62	13537	0.39	4.63	91.42	adjacent to cattle field, high turbidity
4	Billings	7.27	363.0	0.41	38	0.31	3.66	89.52	downhill from cattle fields
7	Cross Timbers	9.56	59.86	1.24	290	0.30	7.33	58.70	previously adjacent to wheat and alfalfa, currently used for cattle grazing
11	Hermitage	8.72	162.3	1.90	518	0.29	2.35	86.68	blue-green dye used, adjacent to cattle field
17	Lamar	9.52	273.6	1.38	12292	0.42	2.08	70.28	adjacent to corn, wheat, and fescue grass
18	Lamar	9.12	92.38	0.65	14139	0.46	4.52	95.50	adjacent to soybean and corn
19	Urbana	9.83	181.5	--	3920	--	--	--	sediment unable to be collected due to rocky/gravel bottom
20	Urbana	9.39	182.9	1.03	5619	0.20	3.16	82.88	adjacent to cattle field

Ib. Descriptive information for each conservation (C) and golf course (G) pond including site number, location, average water (pH, Cond = conductivity (μS cm^{-1}), depth (m), and SArea = open water area (ha)) and sediment (Atz = atrazine concentrations (ng g^{-1}), OM = organic matter content (%), clay and silt (%)) and sediment variables, and field notes.

			Water variables				Sediment variables			
Site	**City**	**Pond category**	**pH**	**Cond**	**Depth**	**SArea**	**Atz**	**OM**	**Clay and silt**	**Field Notes**
5	Kirbyville	C	6.13	46.16	0.40	11	0.29	7.75	85.20	heavy tree canopy cover, *Lespedeza cuneata*
6	Aurora	G	7.05	195.3	0.55	45	0.34	4.44	86.62	geese in pond, heavy grounds management
8	Neosho	G	6.54	50.64	2.05	180	0.41	3.43	92.08	grounds converted to track/field course
9	Neosho	G	7.22	103.2	0.75	87	0.35	4.59	85.46	grounds converted to track/field course, urbanized
10	Neosho	G	7.29	87.10	1.76	336	0.36	2.83	87.50	grounds converted to track/field course, urbanized, *L. cuneata* and *Lonicera japonica*
12	Springfield	G	8.76	598.8	0.73	1097	0.23	5.81	87.34	heavily vegetated
13	Kirbyville	C	6.91	90.58	0.20	253	0.18	12.47	85.56	pond bottom covered in decomposing leaves
14	Kirbyville	C	6.60	310.6	0.25	14	0.80	8.77	88.76	between food plots, amphibians present
15	Kirbyville	C	6.65	138.5	0.31	5	0.21	4.39	81.94	in the middle of a food plot
16	Kirbyville	C	6.48	70.14	0.21	12	0.25	8.45	79.86	on the side of the main road

Appendix II. Macrophyte Survey Data: Species Cover

IIa. Macrophyte and algae cover data in agricultural (A) ponds.

Macophyte	**A1**	**A2**	**A3**	**A4**	**A7**	**A11**	**A17**	**A18**	**A19**	**A20**
Ceratophyllum demersum	28.75	0	0	0	0	0	0	29	58.25	7
Cladophora sp.	39.7	0	32.5	0	0	0	62.25	24	0	37.75
Eleocharis quadrangulata	0	0	0	0	0	0	0	0	0	0
Lemna minor	0	0	0	70	0	0	0	0	0	0
Ludwigia palustris	0	1.75	0	0	0	0	0	0	0	0.25
Ludwigia peploides	0	0	0	0	0	0	0	14	0	0
Myriophyllum sp.	0	0	0	0	0	0	0	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	0
Myriophyllum spicatum	0	0	0	0	0	0	0	0	0	3
Nelumbo lutea	0	0	0	0	0	0	0	0	29.25	6.5
Potamogeton sp.	0	0	0	0	0	0	0	0	0	7.25
Potamogeton diversifolius	0	0	0	0	0	0	0	0	15.5	0
Potamogeton foliosus	0	0	0	21.75	0	0	0	0	0	0
Persicaria punctata	0	0	0	0	0	0	0	0	0	0
Spirodela polyrhiza	0	0	0	0	2	0	0	2	0	0
Spirogyra spp.	0	0	0	0	0	0	0.25	0	18.75	0
Wolffia sp.	0	0	0	21.25	0	0	0	0	0	0
Wolffia brasiliensis	0	0	0	0	77.25	0	0	0	0	0
Zannichellia palustris	0	0.5	0	4.5	0.75	0	0	0	0	0

IIb. Macrophyte and algae cover data in conservation (C) and golf course (G) ponds.

Macophyte	**C5**	**C13**	**C14**	**C15**	**C16**	**G6**	**G8**	**G9**	**G10**	**G12**
Ceratophyllum demersum	25.25	0	61.5	0	0	1	0	0	0	27.75
Cladophora sp.	0	0	0	0	0	0	0	0	0	17.25
Eleocharis quadrangulata	0	0	0	0	11	0	0	0	0	0
Lemna minor	0	29	90.5	97.5	53.75	0	0	0	0	0
Ludwigia palustris	0	0	0	0	0	17	0	0	0	0
Ludwigia peploides	0	0	0	0	0	0	10.75	1.75	0	0
Myriophyllum sp.	0	0	0	0	0	0	31	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	63.25
Myriophyllum spicatum	0	0	0	0	0	0	0	0	0	0
Nelumbo lutea	0	0	0	0	0	0	0	0	0	0
Potamogeton sp.	0	0	0	0	0	0	0	0	0	0
Potamogeton diversifolius	0	0	0	0	0	0	0	0	0	0
Potamogeton foliosus	0	0	0	0	0	0	0	35	74.25	0
Persicaria punctata	0	0.5	0	0	1.5	0	0	0	0	0
Spirodela polyrhiza	97.5	54.5	0	0	20.5	0	0	0	0	0
Spirogyra spp.	0	0	0	0	0	38	68.5	6	0	0
Wolffia sp.	37.5	0	0	0	11	0	0	0	0	0
Wolffia brasiliensis	0	2.5	0	0	0	0	0	0	0	1
Zannichellia palustris	0	0	0	0	0	0	0	0	0	0

Appendix III. Macrophyte Survey Data: Species Composition

IIIa. Macrophyte and algae composition data in agricultural (A) ponds.

Macophyte	**A1**	**A2**	**A3**	**A4**	**A7**	**A11**	**A17**	**A18**	**A19**	**A20**
Ceratophyllum demersum	5.25	0	0	0	0	0	0	4.20	4.78	1.13
Cladophora sp.	7.25	0	20	0	0	0	9.96	3.48	0	6.11
Eleocharis quadrangulata	0	0	0	0	0	0	0	0	0	0
Lemna minor	0	0	0	5.96	0	0	0	0	0	0
Ludwigia palustris	0	7.70	0	0	0	0	0	0	0	0.04
Ludwigia peploides	0	0	0	0	0	0	0	2.03	0	0
Myriophyllum sp.	0	0	0	0	0	0	0	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	0
Myriophyllum spicatum	0	0	0	0	0	0	0	0	0	0.49
Nelumbo lutea	0	0	0	0	0	0	0	0	2.40	1.05
Potamogeton sp.	0	0	0	0	0	0	0	0	0	1.17
Potamogeton diversifolius	0	0	0	0	0	0	0	0	1.27	0
Potamogeton foliosus	0	0	0	1.85	0	0	0	0	0	0
Persicaria punctata	0	0	0	0	0	0	0	0	0	0
Spirodela polyrhiza	0	0	0	0	0.25	0	0	0.90	0	0
Spirogyra spp.	0	0	0	0	0	0	0.04	0	1.54	0
Wolffia sp.	0	0	0	1.81	0	0	0	0	0	0
Wolffia brasiliensis	0	0	0	0	9.66	0	0	0	0	0
Zannichellia palustris	0	2.20	0	0.38	0.09	0	0	0	0	0

IIIb. Macrophyte and algae composition data in conservation (C) and golf course (G) ponds.

Macophyte	**C5**	**C13**	**C14**	**C15**	**C16**	**G6**	**G8**	**G9**	**G10**	**G12**
Ceratophyllum demersum	1.58	0	4.05	0	0	0.18	0	0	0	2.54
Cladophora sp.	0	0	0	0	0	0	0	0	0	1.58
Eleocharis quadrangulata	0	0	0	0	1.13	0	0	0	0	0
Lemna minor	0	3.35	5.95	10	5.5	0	0	0	0	0
Ludwigia palustris	0	0	0	0	0	3.09	0	0	0	0
Ludwigia peploides	0	0	0	0	0	0	0.98	0.41	0	0
Myriophyllum sp.	0	0	0	0	0	0	2.81	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	5.79
Myriophyllum spicatum	0	0	0	0	0	0	0	0	0	0
Nelumbo lutea	0	0	0	0	0	0	0	0	0	0
Potamogeton sp.	0	0	0	0	0	0	0	0	0	0
Potamogeton diversifolius	0	0	0	0	0	0	0	0	0	0
Potamogeton foliosus	0	0	0	0	0	0	0	8.19	10	0
Persicaria punctata	0	0.06	0	0	0.15	0	0	0	0	0
Spirodela polyrhiza	6.08	6.30	0	0	2.10	0	0	0	0	0
Spirogyra spp.	0	0	0	0	0	0	0	6.21	1.40	0
Wolffia sp.	23.4	0	0	0	1.13	0	0	0	0	0
Wolffia brasiliensis	0	2.89	0	0	0	0	0	0	0	0.09
Zannichellia palustris	0	0	0	0	0	0	0	0	0	0

Appendix IV. Macrophyte Survey Data: Species Frequency

IVa. Macrophyte and algae frequency data in agricultural (A) ponds.

Macophyte	**A1**	**A2**	**A3**	**A4**	**A7**	**A11**	**A17**	**A18**	**A19**	**A20**
Ceratophyllum demersum	75	0	0	0	0	0	0	70	90	40
Cladophora sp.	50	0	60	0	0	0	90	60	0	80
Eleocharis quadrangulata	0	0	0	0	0	0	0	0	0	0
Lemna minor	0	0	0	90	0	0	0	0	0	0
Ludwigia palustris	0	20	0	0	0	0	0	0	0	10
Ludwigia peploides	0	0	0	0	0	0	0	30	0	0
Myriophyllum sp.	0	0	0	0	0	0	0	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	0
Myriophyllum spicatum	0	0	0	0	0	0	0	0	0	20
Nelumbo lutea	0	0	0	0	0	0	0	0	30	20
Potamogeton sp.	0	0	0	0	0	0	0	0	0	50
Potamogeton diversifolius	0	0	0	0	0	0	0	0	50	0
Potamogeton foliosus	0	0	0	60	0	0	0	0	0	0
Persicaria punctata	0	0	0	0	0	0	0	0	0	0
Spirodela polyrhiza	0	0	0	0	80	0	0	30	0	0
Spirogyra spp.	0	0	0	0	0	0	10	0	40	0
Wolffia sp.	0	0	0	50	0	0	0	0	0	0
Wolffia brasiliensis	0	0	0	0	100	0	0	0	0	0
Zannichellia palustris	0	20	0	80	30	0	0	0	0	0

IVb. Macrophyte and algae frequency data in conservation (C) and golf course (G) ponds.

Macophyte	**C5**	**C13**	**C14**	**C15**	**C16**	**G6**	**G8**	**G9**	**G10**	**G12**
Ceratophyllum demersum	100	0	90	0	0	40	0	0	0	70
Cladophora sp.	0	0	0	0	0	0	0	0	0	70
Eleocharis quadrangulata	0	0	0	0	60	0	0	0	0	0
Lemna minor	0	100	100	100	100	0	0	0	0	0
Ludwigia palustris	0	0	0	0	0	70	0	0	0	0
Ludwigia peploides	0	0	0	0	0	0	50	20	0	0
Myriophyllum sp.	0	0	0	0	0	0	100	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	70
Myriophyllum spicatum	0	0	0	0	0	0	0	0	0	0
Nelumbo lutea	0	0	0	0	0	0	0	0	0	0
Potamogeton sp.	0	0	0	0	0	0	0	0	0	0
Potamogeton diversifolius	0	0	0	0	0	0	0	0	0	0
Potamogeton foliosus	0	0	0	0	0	0	0	70	80	0
Persicaria punctata	0	20	0	0	60	0	0	0	0	0
Spirodela polyrhiza	100	100	0	0	100	0	0	0	0	0
Spirogyra spp.	0	0	0	0	0	70	90	50	0	0
Wolffia sp.	100	0	0	0	100	0	0	0	0	0
Wolffia brasiliensis	0	100	0	0	0	0	0	0	0	40
Zannichellia palustris	0	0	0	0	0	0	0	0	0	0

Appendix V. Macrophyte and Algae Data: Species Distribution

Va. Macrophyte and algae atrazine resistance and presence, by sampling site, in agricultural (A) ponds.

Macrophyte	Atrazine resistant	Sampling site
Apocynum sp.		A: 7, 11
Carduus nutans		A: 17, 19
Carex frankii		A: 17, 19
Carex grayii		A: 11
Ceratophyllum demersum	X	A: 1, 5, 18, 19, 20
Chara sp.		A: 18, 20
Cicuta sp.		A: 19
Cladophora sp.		A: 17, 19
Eleocharis obtusa		A: 11, 20
Hordeium jubatum		A: 18
Leersia oryzoides	X	A: 19, 20
Lemna minor	X	A: 4
Ludwigia palustris		A: 2, 20
Ludwigia peploides	X	A: 18
Myriophyllum spicatum	X	A: 20
Nelumbo lutea		A: 19, 20
Persicaria punctatum	X	A: 1, 2, 3, 7, 17, 18, 19, 20
Phalaris arundinacea	X	A: 1
Potamogeton diversifolius		A: 19
Potamogeton foliosus		A: 4
Potamogeton nodosus		A: 18
Potamogeton pusillus		A: 20
Rumex crispus		A: 2, 3, 11, 17
Salix nigra	X	A: 3, 11, 17, 18, 19, 20
Scirpus cyperinus		A: 11
Setaria viridis	X	A: 11, 17, 18
Spirodela polyrhiza		A: 7, 18
Spirogyra spp.	X	A: 1, 3, 17, 18, 20
Typha latifolia	X	A: 7
Wolffia sp.	X	A: 4
Wolffia brasiliensis	X	A: 7, 18
Zannichellia palustris		A: 2, 4, 7

Vb. Macrophyte and algae atrazine resistance and presence, by sampling site, in conservation (C) and golf course (G) ponds.

Macrophyte	Atrazine resistant	Sampling site
Acer saccharium		G: 8, 9
Apocynum sp.		G: 8, 10
Arundo donax		G: 12
Ceratophyllum demersum	X	C: 5, 14; G: 6, 12
Chasmanthium latifolium		C: 16
Cladophora sp.		G: 6, 8, 9
Eleocharis obtusa		C: 15; G: 9, 10
Eleocharis quadrangulata		C: 16, 19; G: 8
Elymus elymoides		C: 14, 16
Elymus jejunes		C: 14; G: 12
Fraxinus sp.		C: 13
Hordeium jubatum		C: 14
Juglans nigra		G: 12
Justica americana		G: 12
Leersia oryzoides	X	C: 16
Lemna minor	X	C: 13, 14, 15, 16
Lonicera japonica		G: 10
Ludwigia palustris		G: 6
Ludwigia peploides	X	G: 8, 9
Luzula sp.		C: 14
Myriophyllum sibiricum	X	G: 8, 12
Persicaria punctatum	X	C: 13, 15, 16; G: 12
Potamogeton foliosus		G: 9, 10
Rumex crispus		G: 12
Salix nigra	X	G: 8, 10
Schoenoplectus americanus		C: 16
Scirpus cyperinus		G: 9, 10
Setaria viridis	X	C: 15; G: 9
Spirodela polyrhiza		C: 5, 13, 16
Spirogyra spp.	X	G: 12
Typha latifolia	X	G: 9, 10
Ulmus sp.		C: 13; G: 8, 10
Wolffia sp.	X	C: 5, 16
Wolffia brasiliensis	X	C: 13; G: 12