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Water quality, habitat, and fish assemblage relationships in middle-order agriculture and forest streams of the Mississippi Alluvial Plain

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Abstract

Background Agriculture has greatly influenced water quality, habitats, and fish assemblages in streams of the Mississippi Alluvial Plain (MAP) ecoregion. However, MAP streams have historically been understudied compared to streams in other agricultural regions of the USA. In this study, water quality, habitat, and fish assemblage composition were assessed seasonally (spring, summer, and fall) in eight representative MAP streams located across three U.S. states. The study design included four streams containing highly agricultural watersheds (herein termed "agriculture" streams) and four streams containing mostly forested watersheds (herein termed "forest" streams), which were intended to represent reference conditions for MAP streams.

Results In general, forest streams contained significantly better instream and riparian habitats than agriculture streams ($P = 0.010$–0.040) whereas agriculture streams contained significantly greater levels of primary nutrients ($P < 0.001$–0.010). Differences between agriculture and forest streams with respect to other physical and chemical variables were intermittent and season dependent. Fish assemblages in agriculture and forest streams were structured primarily along an environmental gradient reflecting instream habitat conditions, water nutrient concentrations, and benthic chlorophyll-*a* production. Structurally, fish assemblages in both stream types contained many regionally common species, though some species appeared to exhibit affinities for a particular stream type. Functionally, fish assemblages in agriculture streams contained more tolerant species, more omnivores, and fewer insectivores compared to forest stream assemblages, which were nearly all insectivores. Overall, one-third of the fish specimens collected in forest streams classified as intolerant species.

Conclusions Our results suggested that stream water quality, habitat, and fish assemblages differed between agriculture and forest streams in the MAP, with fish assemblages exhibiting both structural and functional differences. Results were consistent with a larger body of literature from smaller, headwater streams whereby land-use changes (e.g., row-crop agriculture) impacted the physical, chemical, and biological characteristics of stream ecosystems. Results further highlight the importance of land use management and its effects on habitat diversity in stream ecosystems, and that protecting the few remaining undisturbed or less-disturbed streams should be a priority.

Keywords Agriculture, Stream, Land use, Water quality, Habitat, Fish, Multivariate statistics, Mississippi Alluvial Plain

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Background

Row-crop agriculture is the single most pervasive anthropogenic influence that has affected streams and small rivers throughout the Mississippi Alluvial Plain (MAP) ecoregion (Justus 2003; Wentz et al. 2011; Shrestha et al. 2017; Robison and Buchanan 2020). Jelks et al. (2008) reported that up to 34 imperiled fish species reside in the Mississippi Embayment physiographic region, which includes the entire MAP ecoregion. Species imperilment in these systems is often a function of flow alteration, water quality declines, and habitat degradation (Warren et al. 2000; Wang et al. 2006). In terms of water quality, row-crop agriculture commonly increases loads of suspended sediments (Culp et al. 2013; Meador and Frey 2018) and nutrients (Jordan et al. 1997; Schilling and Libra 2000; Turunen et al. 2019; Taylor et al. 2023). Similarly, many agricultural practices decrease instream habitat conditions through both habitat homogenization (e.g., channelization) (Walser and Bart 1999) and/or replacement of natural habitats with anthropogenic structures such as tires, furniture, large appliances, and other materials (Wentz et al. 2011). The chemical and physical characteristics of stream ecosystems, in part, determine the composition of the resident species assemblages (Tonn 1990; Poff 1997; Marsh-Matthews and Matthews 2000). Because row-crop agriculture can greatly alter the physical and chemical characteristics of stream ecosystems, stream fish assemblages are often directly and indirectly influenced by agricultural activities occurring adjacent to streams (Shields et al. 1995; Rowe et al. 2009; Turunen et al. 2019; Alvarenga et al. 2021). In the MAP ecoregion alone, row-crop agriculture has affected many hundreds of streams in the aforementioned manners (Ross 2001; Robison and Buchanan 2020).

Row-crop agriculture is widespread throughout the MAP, as are agricultural effects on the physical and chemical environments of receiving streams. Justus (2003) estimated that over 70% of the total land in the MAP is dedicated to the production of corn, soybeans, cotton, and rice, with over 90% of some watersheds dedicated exclusively to production of these crops (McCarthy et al. 2012). To facilitate row-crop production, major alterations to MAP streams have occurred, including construction of levee networks to restrict flooding, draining of natural wetlands for conversion to croplands, straightening of stream channels for more efficient tillage, and clearing of native forests (Justus 2003; McCarthy et al. 2012; Shrestha et al. 2017). All of these practices in concert have degraded instream habitat conditions and water quality for aquatic biota (Wentz et al. 2011). As a result, most MAP streams are typified by elevated phosphorus concentrations (Giese et al. 1987; Wentz et al. 2011; Shields et al. 2013), and excessive turbidities and suspended solid concentrations during some seasons (Stephens et al. 2008; Wentz et al. 2011; Shields et al. 2013). Flows in some MAP streams are more linked to seasonal runoff from irrigation activities than to seasonal rainfall or groundwater inputs (Shrestha et al. 2017; Yasarer et al. 2020). Seasonally low dissolved oxygen concentrations also have become common in many MAP streams, though low concentrations do occur naturally in some streams during portions of the year (Justus et al. 2012; Chen et al. 2015).

The alteration of stream chemical and physical characteristics related to agriculture can have serious implications for fish assemblages, including decreased biotic integrity (Shields et al. 1995; Roth et al. 1996; Wang et al. 1997; Saalfeld et al. 2012) and decreased abundances of sensitive species (Waite and Carpenter 2000; Warren et al. 2000; Fitzpatrick et al. 2001). Although fish assemblages in the MAP ecoregion were relatively diverse historically (Robison and Buchanan 2020), most streams are now heavily altered and tend to be dominated by moderately to highly tolerant species such as western mosquitofish (*Gambusia affinis*), green sunfish (*Lepomis cyanellus*), blacktail shiner (*Cyprinella venusta*), and red shiner (*Cyprinella lutrensis*) among others (Keith and Shirley 1985; Stephens et al. 2008; Shields et al. 2013; Shrestha et al. 2017; Robison and Buchanan 2020). Relatively undisturbed or less-disturbed streams in the MAP, which are typified by more natural conditions and usually have greater abundances of less tolerant or intolerant species (Keith and Shirley 1985; Killgore and Baker 1996; Buchanan 1997; Robison and Buchanan 2020), are many fewer in number than highly disturbed streams. Although the degree to which MAP streams have been affected by row-crop agriculture is assumed to be pronounced (Wentz et al. 2011; Justus et al. 2012), these streams have historically been understudied compared to other types of streams. Logistical factors such as lack of access due to remoteness, large-scale private property issues, and intermediate sizes (e.g., too small for boats but still not wadeable) create challenges for conducting research. Additionally, from an experimental design perspective, agricultural effects are so widespread in the MAP that it is often difficult to locate appropriate reference streams for comparisons.

In light of the above characteristics for MAP streams, the number of imperiled fishes contained in these systems, and the relative scarcity of previous research, ecological studies assessing agricultural effects in comparison to streams typifying reference conditions are warranted. Our objectives in this study were to: (1) quantify differences in selected physical, chemical, and fish-assemblage variables between streams heavily influenced by agriculture and nearby reference streams, and

(2) assess structural and functional differences between fish assemblages in streams heavily influenced by agriculture and nearby reference streams. This study emphasized third-order and fourth-order streams to enhance a growing body of literature that has focused more on smaller, headwater systems (e.g., Smiley et al. 2009, 2017; Shrestha et al. 2017; Sanders et al. 2020). Reference streams used in this study were considered to be the closest possible representations of MAP stream ecosystems prior to agricultural development. Although there was some subjectivity in these estimations, these streams contained mostly forested watersheds, were relatively close to agriculturally influenced streams (i.e., most < 100 km away), and were of comparable sizes. Further knowledge emphasizing relationships among physical, chemical, and biota in agricultural streams will enhance understanding of the ecology of MAP streams.

Methods

Study areas

Streams in the MAP ecoregion tend to be low gradient, have variable ill-defined channels, silt-dominated substrates, and instream habitats consisting largely of brush, logs, vegetation, and other organic matter (Giese et al. 1987). Watersheds were historically dominated by bottomland hardwood forests and forested wetlands, with some scattered grass prairies. The mass conversion of the MAP to large-scale, row-crop agriculture began around 1830 (Gatewood 1991), with water withdrawals for irrigation-based agriculture becoming more intense after 1900 (Yasarer et al. 2020). Conversely, small-scale (< 20 ha) subsistence agriculture was largely restricted to more upland areas adjacent to the MAP that flooded less frequently.

This study focused on eight third-order and fourth-order MAP streams located across an approximately 6,000 km^2 area in Arkansas, Louisiana, and Mississippi, USA (Fig. 1). These study streams were selected from a larger pool of available third-order and fourth-order streams and considered representative of MAP streams. Four study streams were selected that contained highly agricultural watersheds (herein termed “agriculture” streams) and four nearby streams were selected that contained largely forested watersheds (herein termed “forest” streams). Sampling locations for all forest streams were located within the White River National Wildlife Refuge, though portions of their watersheds were outside the refuge and not completely forested. Within this assessment, characteristics in forest streams were intended to reflect reference conditions in the MAP for comparison to those in nearby agriculture streams. Land cover in agriculture streams averaged 66 ± 17% (mean ± SD) cultivated croplands while forest streams were 17 ± 13% cultivated croplands (Homer et al. 2015). In all eight streams, three sites located in upper, middle, and lower reaches were chosen for assessment, with each site sampled once each during spring (April), summer (June), and fall (October) of 2014. Overall, a total of 24 sites were sampled across the eight streams used in this study. Assessments entailed water quality, chlorophyll-*a* (chl-*a*), fish assemblages, and instream habitats. On occasion, alternative sites were selected during low-water periods in fall. Appendix A contains the protocol for our selection of alternate sites when they were needed.

Physical and chemical measurements

A total habitat score was computed for each site during each sampling following the U.S. Environmental Protection Agency’s Rapid Bioassessment Protocol for Wadeable Streams and Rivers (low-gradient modification; Barbour et al. 1999). This habitat score is composed of a suite of measures that reflect the condition of both instream and riparian habitats. Specifically, the index encompasses on-site visual assessments of ten key habitat indicators that reflected: substrate suitability for epifaunal colonization, pool substrate characterization, variability in pool sizes, degree of sediment deposition, degree of channel bed exposure, degree of channel alteration, channel sinuosity, overall stream bank stability, extent of vegetative cover on stream banks, and riparian zone width. Each indicator was scored visually on a 1–20 scale that reflected poor (0–5), marginal (6–10), suboptimal (11–15), and optimal (16–20) conditions. Indicator scores were then summed into an overall total habitat score categorized as poor (0–50), marginal (51–100), suboptimal (101–150), and optimal (151–200). Additionally, the first six indicators listed above were collapsed into a single score that reflected instream habitat condition whereas the last four scores were collapsed into a single score that reflected riparian habitat conditions. This methodology was used by Shrestha et al. (2017) in similar MAP streams,

Water temperature (°C), dissolved oxygen (DO, mg/L), specific conductance (μS/cm), and salinity (ppt) were measured and recorded in the field with a portable multiprobe field meter (Model: YSI 85-25FT). Water pH (standard units) was measured using a portable pH meter (Model: Ecosense pH 10A). Turbidity (as NTU) was measured with a portable turbidity field meter (Model: Hach 2100P). Water samples (2-L) were collected in acid-washed plastic bottles and transported on ice to the laboratory for analysis. Additional water chemistry variables such as total suspended solids (TSS, mg/L), total alkalinity (mg/L as $CaCO_3$), chloride (mg/L), total hardness (mg/L as $CaCO_3$), total ammonia nitrogen (TAN, mg/L), nitrate (mg/L), phosphate (mg/L), total nitrogen (mg/L),

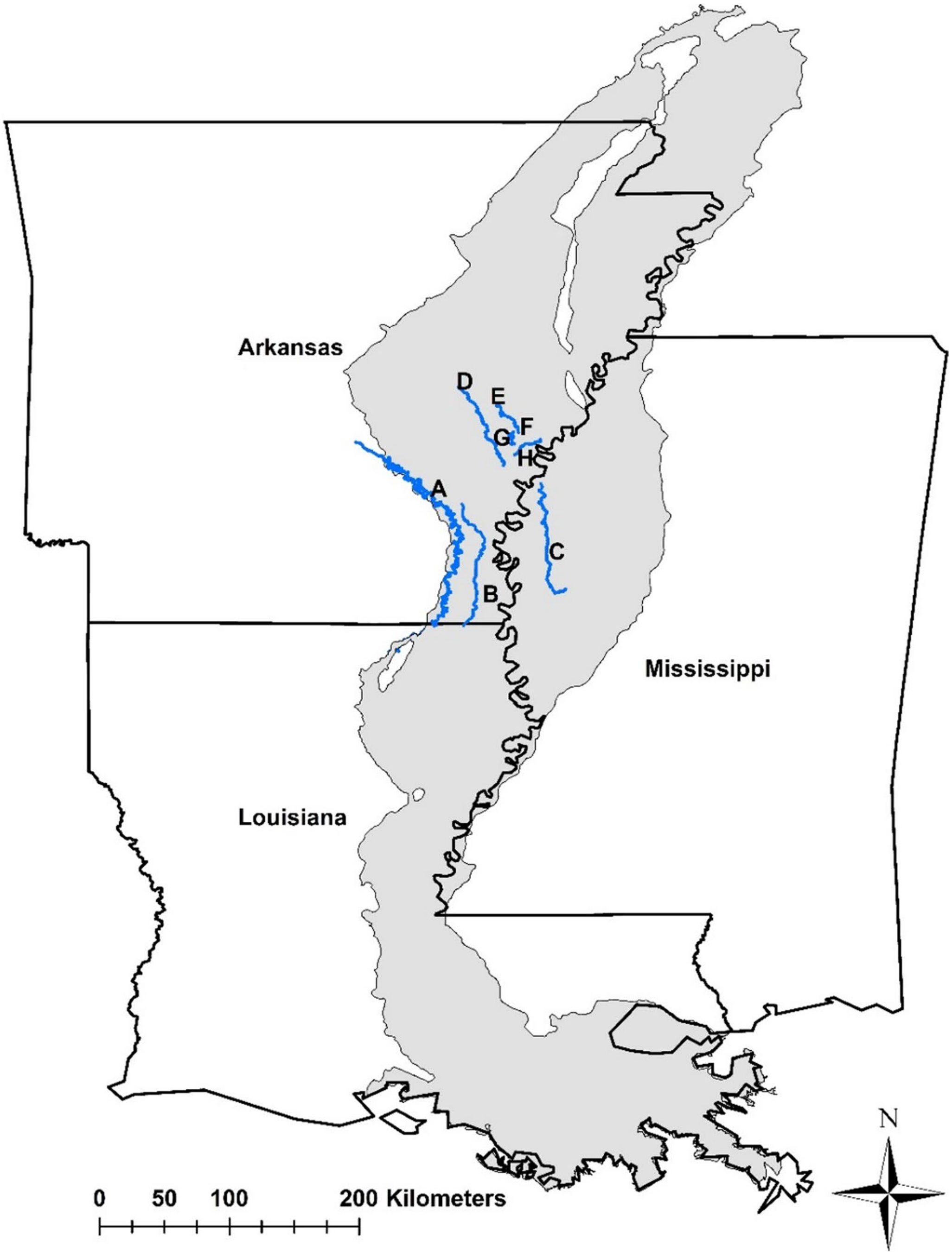

Fig. 1 Locations of streams used in this study within the Mississippi Alluvial Plain, USA. The specific streams are labeled as **A** Bayou Bartholomew, Arkansas (order 3–4), **B** Bayou Beouf, Arkansas-Louisiana (order 4), **C** Bouge Philia, Mississippi (order 4), **D** LaGrue Bayou, Arkansas (order 3–4), **E** Maddox Bay, Arkansas (order 3), **F** Indian Bay, Arkansas (order 3), **G** Big Island Chute, Arkansas (order 3), and **H** Cypress Bayou, Arkansas (order 3). **A**, **B**, **C** and **H** represent agriculture streams while **D**, **E**, **F**, and **G** represent forest streams. The gray region delineates the Mississippi Alluvial Plain ecoregion

and total phosphorus (mg/L) were measured in the laboratory following APHA (2005). All water quality samples were taken within the sampling site.

Two different measures of chl-*a* were collected at all sites. Benthic chl-*a* concentrations as reflected by benthic algae samples were collected in accordance with

Hauer and Lamberti (2007) using modified procedures for silt-dominated substrates. Three 3-cm diameter sediment core samples were taken at each site during each season, with the upper 1 cm of each core removed. Core samples were combined to form a composite sample for each stream, with each sample kept refrigerated in a dark container to limit post-collection primary production. Benthic chl-*a* concentrations were determined by first adding 10 mL of 95% ethanol solution to preserve the chlorophyll-*a* concentrations, with samples refrigerated afterwards at 4 °C for 24 h. Following refrigeration, samples were centrifuged for 10 min, with chl-*a* concentrations measured with a bench-top spectrophotometer using an acidification process (0.1-M hydrochloric acid added to correct for pheophytin presence) (Barbour et al. 1999; Hauer and Lamberti 2007). Water-column chl-*a* as reflected by suspended algae (i.e., phytoplankton) was taken from the same water samples collected for water quality as described previously. Depending on the expected concentration in the sample, a subsample was collected from water samples and filtered through a 0.45-μm glass filter (Whatman) to remove the phytoplankton. Filters were then centrifuged in tubes with 10 mL of 95% ethanol solution and refrigerated afterwards at 4 °C for 12 h. Samples were then analyzed for chl-*a* concentrations using the same process described for benthic chl-*a*. Overall, this study quantified 18 environmental variables in total—14 water quality, two chl-*a*, one physical, and one habitat.

Fish collections and assemblage measures

Fish assemblages in MAP streams were sampled using two different passive sampling gears—mini-fyke nets and trap nets. A single net of each type was fished concurrently overnight for 18–24 h at each site during each season. Nets were set in tandem at the same sites, with each net type set on opposite banks to avoid gear saturation or interference issues. Mini-fyke nets contained a 3-mm mesh size and were purchased from a commercial supplier. Net frames were composed of two 7.9-mm-thick oil-tempered steel rectangles with dimensions of 0.6 m × 1.2 m, with a 4.5 m long × 0.6 m high lead that anchored the net to the shore. These nets were identical to those used by Clark et al. (2007) and Lubinski et al. (2008) in nearby streams. The trap nets used contained two rectangular frames (0.9 m high × 1.8 m wide, spaced 0.76 m apart) followed by four 0.8-m diameter hoops spaced 0.6-m apart. Nets contained 1.3-cm bar-mesh and were equipped with 15.0-m long × 1.0-m high lead lines. This style of trap net is a standard design used by many management agencies for indexing warmwater fishes in standing waters (Miranda and Boxrucker 2002). For both gears, collected fishes that were readily identifiable in the field were classified to species, counted, and released alive. Fishes that could not be identified in the field (e.g., juvenile lepomids and cyprinids) were preserved in 10% formalin and returned to the laboratory for identification using standard taxonomic keys (Pflieger 1997; Ross 2001; Robison and Buchanan 2020).

Following collection, laboratory processing, and identification of fishes, several common assemblage measures were calculated from combined samples for each stream type (i.e., all three sites within a stream pooled during each season). Species richness (*S*) was tabulated as the total number of species collected, with an adjusted species richness index calculated as *S* divided the square root of the number of specimens in the sample (*N*). Species diversity was calculated using the Shannon–Wiener diversity index (*H′*) as:

$$H' = -\sum_{i=1}^{s}\left(\frac{n_i}{N}\right)\ln\left(\frac{n_i}{N}\right),$$

where S equals the number of species in the sample, n_i equals the number of individuals of species i, and N is the total number of individuals in the sample (Washington 1984; Hauer and Lamberti 2007). Species evenness was calculated by dividing H' by the theoretical maximum diversity, which was approximated as ln*S* (Hauer and Lamberti 2007). Finally, species dominance (*D*) was calculated using Simpson's index of dominance (*D*) as:

$$D = 1 - \left(\frac{n_i}{N}\right)^2,$$

where n_i equaled the number of species i and N equaled the total number of individuals in the sample. Unlike H', this index places greater emphasis on common rather than rare species, and is scaled from zero (i.e., no diversity) to one (high diversity). During all sampling, water quality, chl-*a*, and habitat assessments were conducted on the same day, with water and chl-*a* samples being taken first followed by habitat assessments; fish assemblages were always sampled last.

Data analysis

When all measurements of water quality, chl-*a*, habitat, and fish-assemblage variables were compiled across streams ($n = 8$), mean values were generated for each variable in each stream ($n = 3$ sites/stream) and season (i.e., spring, summer, fall in each stream; $n = 3$). Thus, seasonal mean values for each variable were generated for each stream from 24 stream–season combinations (i.e., four streams/stream type × three seasons) for use in further analyses. This averaging of sites into a composite stream-specific mean was done to reduce the risk of pseudoreplication and also to buffer potential effects associated with relocation of some of the sampling sites during the fall as outlined in Appendix A.

Under this design, all water quality, chl-*a*, habitat, and fish-assemblage variables measured across seasons were analyzed with repeated-measures analysis of variance (ANOVA) using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina). Seasonal means for each stream averaged across sites were used to test the effects of stream type ($n=2$), season ($n=3$), and season × stream-type interaction ($n=6$) on the variables above. For these analyses, stream type was the main effect, season was the repeated variable, and individual stream nested within stream type (i.e., agriculture or forest) served as the subject. When the season × stream-type interaction was significant, it was interpreted as a difference between stream types, but only during one or two of the three seasons. In these cases, a least-squares means post hoc test was then used in a pairwise fashion to determine which season(s) were driving the interaction. If the season × stream-type interaction was not significant, then the stream type main effect was interpreted directly. If this term was significant, the analysis was interpreted as types were different for the measure in question, and that the difference was consistent seasonally. A significant season effect indicated only that there was consistent temporal variation in the variable being tested for both stream types (this effect was not of interest in this study). Significance for all analyses was declared at an alpha level of 0.05.

Canonical correspondence analysis (CCA; ter Braak 1986; Palmer 1993; Peck 2016) was used to examine relationships between fish assemblage composition and the 18 environmental variables measured from streams. Species that comprised less than 1% of the total catch were eliminated to minimize the possibility that rare species would mask patterns and/or distort the overall CCA (McCune and Grace 2002; Peck 2016). Significance of the fish–environment association was assessed by comparing observed eigenvalues from the first three ordination axes to those generated from randomization of the species × stream data matrix (5000 iterations) using a simple Monte Carlo reshuffling algorithm ($\alpha=0.05$). Environmental variables and species with axis correlations ≤ − 0.3 or ≥ 0.3 were emphasized when interpreting environmental gradients. CCA was conducted using the software PC-ORD V.4 (MjM Software, Gleneden Beach, Oregon, USA).

Results

Physical and chemical variables

Habitat scores overall suggested that agriculture significantly affected the physical features of the receiving streams. Total habitat scores from agriculture streams suggested moderately degraded habitats (150 ± 26) compared to forest streams (182 ± 10), with scores being approximately 20% lower in agriculture streams (Table 1). This trend was mirrored by both instream habitat scores (agriculture: 70 ± 9; forest: 83 ± 9) and riparian habitat scores (agriculture: 80 ± 18; forest: 98 ± 2) (Table 1). Similarly, scores for total habitat ($P=0.020$), instream habitat ($P=0.010$), and riparian habitat ($P=0.004$) were all significantly greater in forest streams compared to agriculture streams (Table 1).

Water quality effects of agriculture on streams were apparent, though effects varied among variables and seasons. Phosphate levels were significantly ($P<0.001$) greater in agriculture streams (0.16 ± 0.08 mg/L) compared to forest streams (0.05 ± 0.03 mg/L) (Table 1). Similarly, total phosphorus concentrations exhibited significantly ($P<0.001$) greater concentrations in agriculture streams (0.47 ± 0.20 mg/L) than forest steams (0.21 ± 0.13 mg/L) (Table 1). Nitrate concentrations were twofold greater in agriculture streams (1.09 ± 0.56 mg/L) compared to forest streams (0.54 ± 0.14 mg/L, $P=0.010$) (Table 1). Total nitrogen concentrations also were significantly ($P=0.001$) greater in agriculture streams (2.52 ± 0.82 mg/L) than forest streams (2.22 ± 0.40 mg/L) (Table 1).

In cases where the season × stream-type interaction term was significant ($P \leq 0.05$), analyses were decomposed such that seasonal differences could be examined. Season × stream-type interaction existed for several variables (Table 1), including specific conductance ($P=0.002$), TAN ($P=0.002$), TSS ($P=0.047$), turbidity ($P=0.045$), pH ($P=0.007$), alkalinity ($P<0.001$), and hardness ($P<0.001$) (Table 1). In particular, specific conductance was significantly greater in agriculture streams, but only during low-flow periods in summer and fall ($P=0.012$–0.244; Table 1, Fig. 2). Similarly, although TAN concentrations were on average sixfold greater in agriculture streams (agriculture: 0.18 ± 0.22 mg/L, forest: 0.03 ± 0.03 mg/L), measures were highly variable, and thus, stream-type differences were only significant during summer ($P<0.001$) (Table 1; Fig. 2). Although both water clarity variables exhibited extreme variation, turbidity ($P=0.002$) and TSS ($P=0.047$) were greater in agriculture streams during higher-flow periods in spring (Table 1, Fig. 3). Conversely, pH ($P=0.021$) and alkalinity ($P=0.028$) were lower in agriculture streams, also only during higher-flow periods in spring (Table 1, Fig. 4). Both of these variables tended to increase seasonally regardless of stream type, with levels being similar in both stream types by summer and fall (Fig. 4). Hardness ($P=0.038$; Table 1, Fig. 5) and TAN ($P<0.001$; Table 1, Fig. 2) were typically greater in agriculture streams, though differences were limited to lower-flow periods in summer and/or fall. Neither stream

Table 1 Repeated-measures ANOVA of environmental (mean ± SD) variables in agriculture and forest streams of the Mississippi Alluvial Plain

Variable	Agriculture	Forest	Season	Main effect (stream type)	Interaction (season × stream type)	
Temperature (°C)	23.32 ± 5.75	21.80 ± 4.60	< 0.001	0.209		0.228
DO (mg/L)	7.00 ± 2.46	6.80 ± 1.14	0.323	0.901		0.111
Specific conductance (μS/cm)	311.79 ± 197.46	231.15 ± 63.22	< 0.001	0.160		**0.002**
					Summer	**0.012**
					Fall	**0.024**
pH (su)	7.96 ± 0.87	7.98 ± 0.41	< 0.001	0.826		**0.007**
					Spring	**0.021**
Turbidity (NTU)	219.08 ± 305.75	49.38 ± 16.10	0.043	0.060		**0.045**
					Spring	**0.002**
Total suspended solids (mg/L)	48.93 ± 44.26	27.35 ± 11.55	0.057	0.117		**0.047**
					Spring	**0.004**
Chloride (mg/L)	21.03 ± 17.83	13.21 ± 8.92	0.005	0.304		0.434
Alkalinity (mg/ L as $CaCO_3$)	93.03 ± 62.54	98.60 ± 26.80	< 0.001	0.750		**< 0.001**
					Spring	**0.028**
Hardness (mg/L as $CaCO_{3)}$	122.53 ± 77.34	105.53 ± 27.72	< 0.001	0.562		**< 0.001**
					Summer	**0.038**
TAN(mg/L)	0.18 ± 0.22	0.03 ± 0.03	0.018	0.005		**0.002**
					Summer	**< 0.001**
Nitrate (mg/L)	1.09 ± 0.56	0.54 ± 0.14	0.269	**0.010**		0.102
Total nitrogen (mg/L)	2.52 ± 0.82	2.22 ± 0.40	0.018	**0.001**		0.212
Phosphate (mg/L)	0.16 ± 0.08	0.05 ± 0.03	0.290	**< 0.001**		0.638
Total phosphorus (mg/L)	0.47 ± 0.20	0.21 ± 0.13	0.003	**< 0.001**		0.678
Water-column Chl-*a* (μg /L)	25.29 ± 18.01	11.83 ± 4.74	0.030	0.087		0.193
Benthic Chl-*a* (μg/cm^3)	0.42 ± 0.27	0.32 ± 0.22	0.002	0.329		0.988
Total Habitat Score	150 ± 26	182 ± 10	0.022	**0.020**		0.372
Instream Habitat Score	70 ± 9	83 ± 9	0.039	**0.010**		0.829
Riparian Habitat Score	80 ± 18	98 ± 2	0.056	**0.040**		0.055

Data were collected in spring, summer, and fall 2014. Significant effects ($P \leq 0.05$) that were interpretable are denoted with bold print. Results for salinity were deemed uninterpretable and are not shown

type nor the interaction of season and stream type significantly affected water-column chl-*a*, benthic chl-*a* concentrations, temperature, DO, or chloride in MAP streams (Table 1). However, estimated concentrations for all of these measures were highly variable as coefficients of variation often exceeded 50%.

Fish assemblages

Across three seasons of fish sampling in these streams, 3535 individual fishes from 13 families encompassing 57 species were collected. In terms of total catch, fishes were consistently more abundant in forest streams, with abundances being 72% greater on average than agriculture streams. Cyprinidae and Centrarchidae dominated fish assemblages in both stream types, comprising 64% and 17% of the specimens collected overall, respectively (Table 2). Centrarchidae (33%) and Cyprinidae (34%) comprised nearly identical proportions of fish assemblages in agriculture streams. Although these two families also dominated fish assemblages in forest streams, the proportions were more skewed toward Cyprinidae (81%) than Centrarchidae (8%) (Table 2).

Fish species richness was similar between stream types, with 47 species collected in forest streams compared to 48 species in agriculture streams; 38 species were common to both stream types (Table 3). Surprisingly, fish assemblage diversity and evenness were generally lower in forest streams. In agriculture streams, H' and E values from combined samples were 3.01 and 0.78, respectively, compared to 2.05 and 0.53, respectively, in forest streams. This driven by the fact that the four most common species comprised 78% of the total catch in forest streams compared to only 46% in agriculture streams (Table 3). The most abundant species in forest streams included emerald shiner (*Notropis atherinoides*), weed shiner (*Notropis texanus*), river shiner (*Notropis blennius*), and

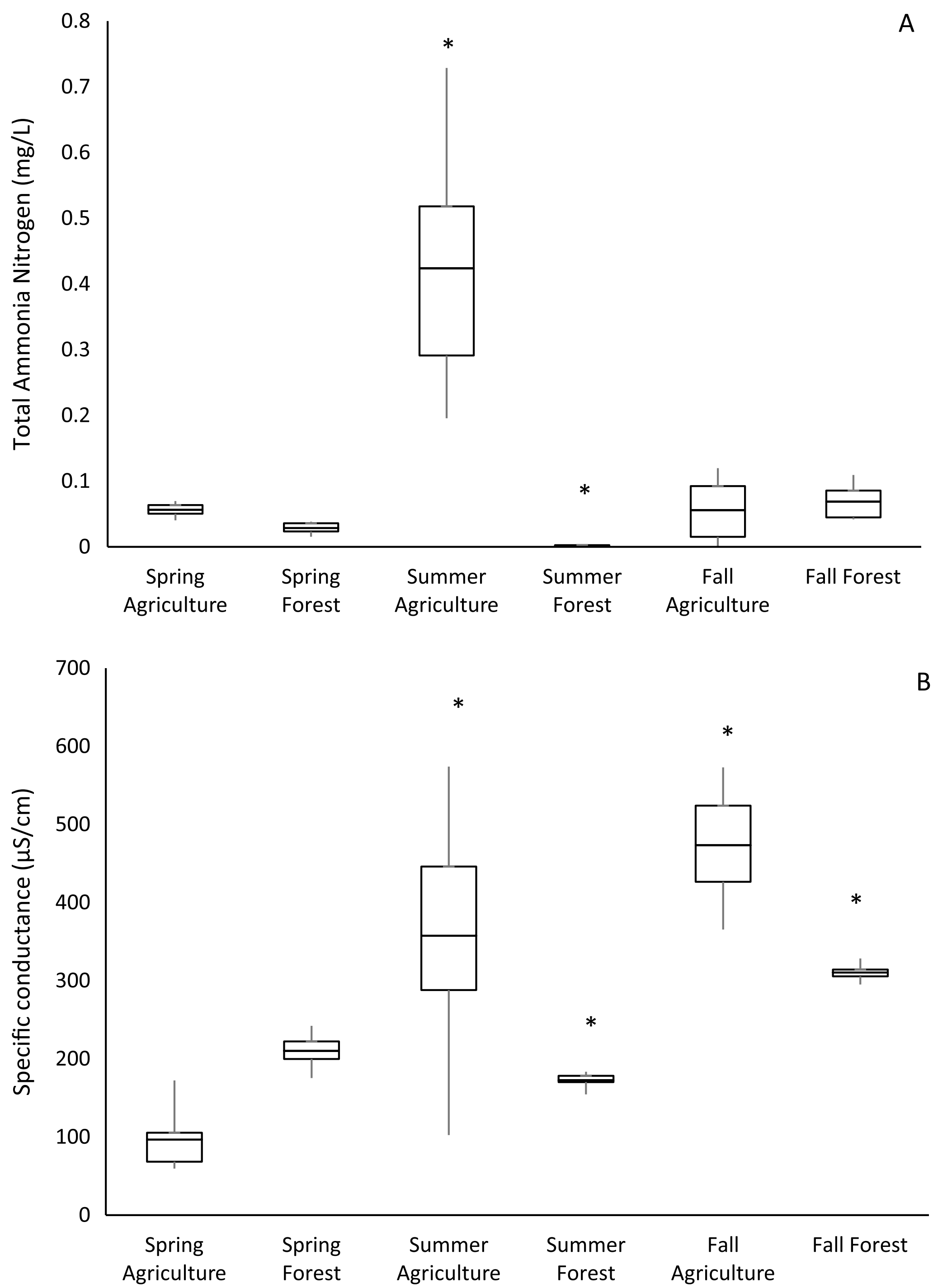

Fig. 2 Seasonal variation in **A** total ammonia nitrogen and **B** specific conductance in agriculture and forest streams of the Mississippi Alluvial Plain. *Indicates significant difference between forest and agriculture streams for that season

bluegill (*Lepomis macrochirus*) (Table 3). The most common species in agriculture streams were pirate perch (*Aphredoderus sayanus*), bluegill, red shiner, orangespotted sunfish (*Lepomis humilis*), river shiner, and western mosquitofish (Table 3). All of these species comprised greater than 4% of the total catch and were relatively common to the region. Interestingly, the only species comprising > 4% of the sample in both stream types was river shiner (Table 3).

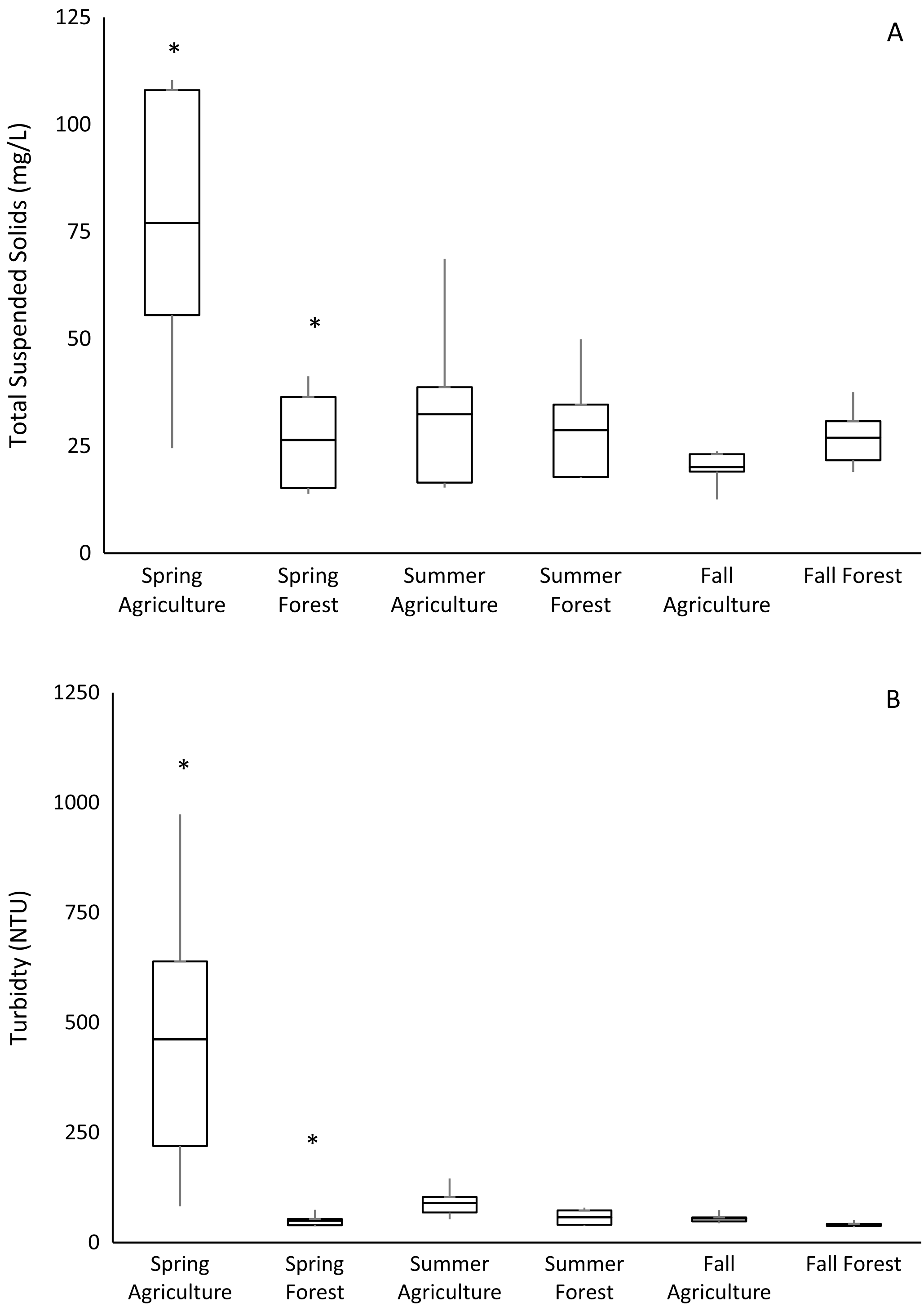

Fig. 3 Seasonal variation in **A** total suspended solids and **B** turbidity in agriculture and forest streams of the Mississippi Alluvial Plain. *Indicates significant difference between forest and agriculture streams for that season

Many fish assemblage metrics exhibited high variation, with SD ranges sometimes including zero. Repeated-measures ANOVA suggested some fish assemblage differences with respect to stream types. The relative abundances of Cyprinidae ($P=0.047$) and Percidae ($P=0.032$) were greater in forest streams compared to

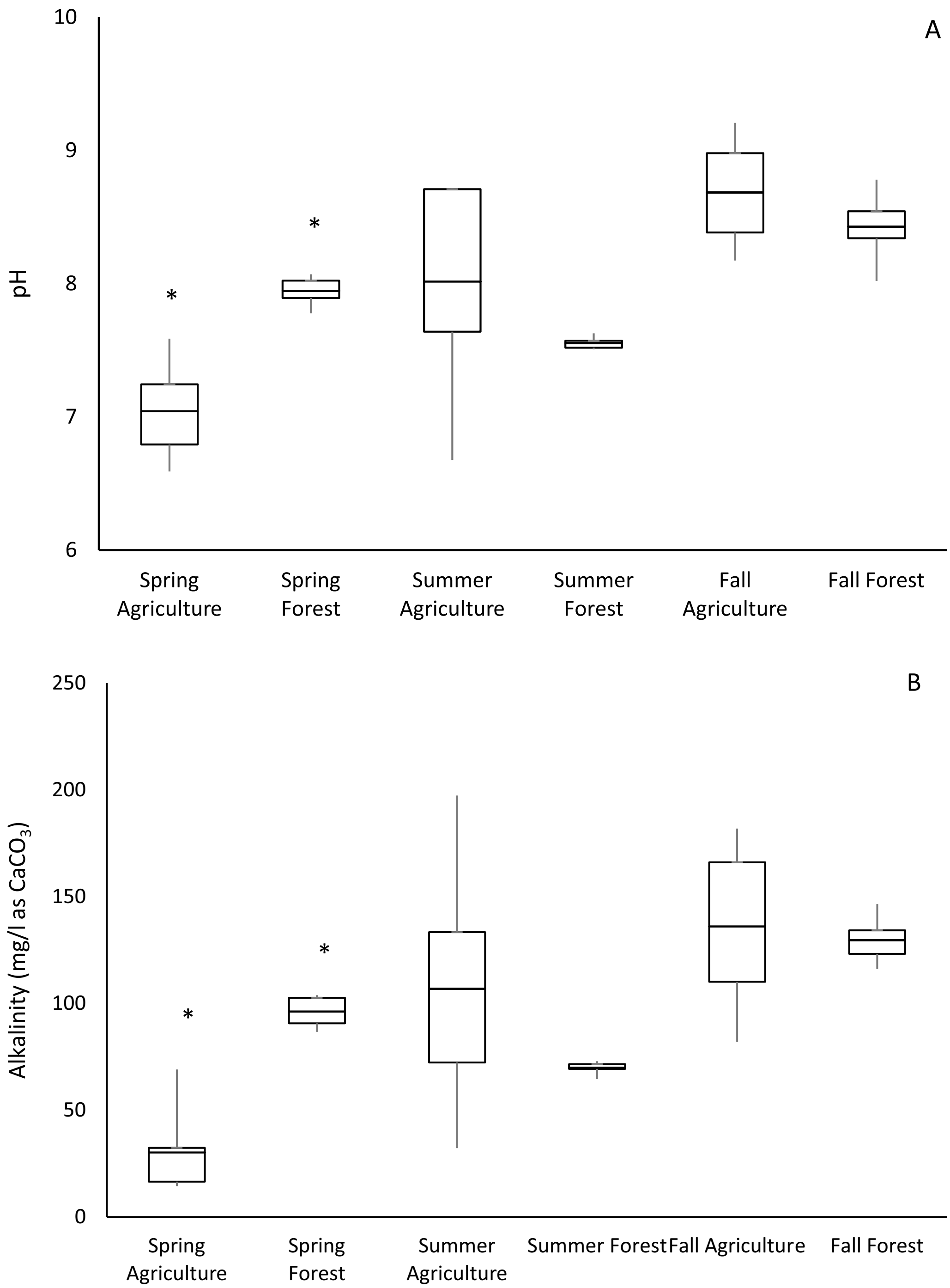

Fig. 4 Seasonal variation in **A** pH and **B** alkalinity in agriculture and forest streams of the Mississippi Alluvial Plain. *Indicates significant difference between forest and agriculture streams for that season

agriculture streams (Table 4). The season × stream-type interaction was significant for relative abundance of intolerant species ($P=0.007$), total fish abundance ($P=0.041$), species richness index ($P=0.017$), Shannon–Wiener diversity ($P=0.034$), and dominance ($P=0.026$) (Table 4). In cases where the season × stream-type interaction term was significant ($P \leq 0.05$), the analysis was decomposed such that seasonal differences could be examined. The relative abundance of intolerant species was significantly greater in forest streams, but only during the spring

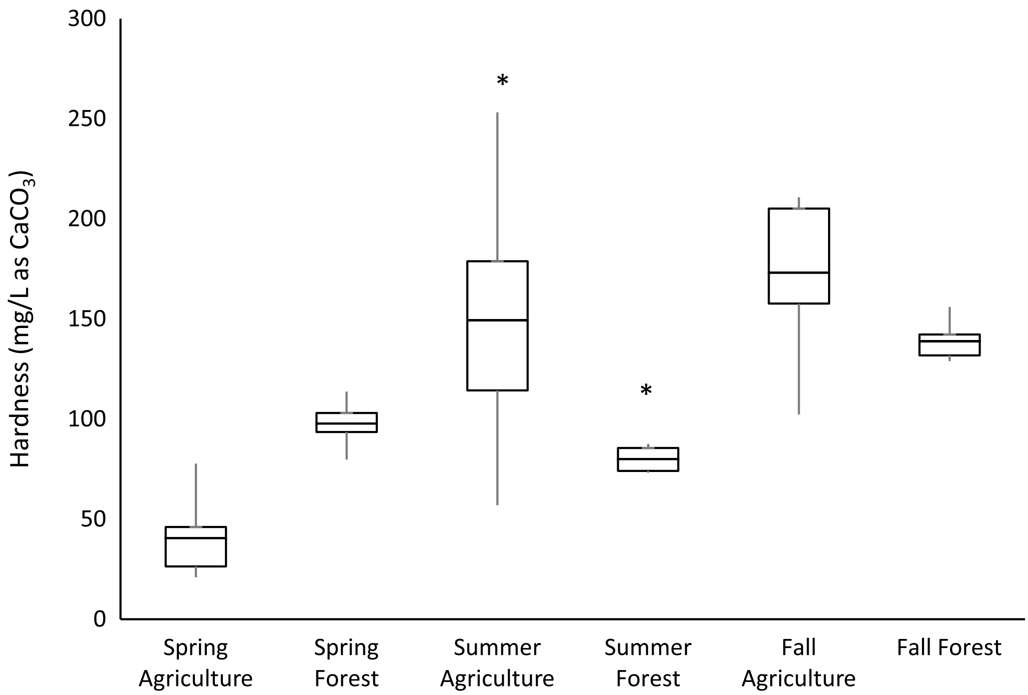

Fig. 5 Seasonal variation in hardness in agriculture and forest streams of the Mississippi Alluvial Plain. *Indicates significant difference between forest and agriculture streams for that season

($P<0.001$) and fall ($P=0.015$) seasons (Table 4). Total fish abundance, though always greater in forest streams, was only significantly greater during fall ($P=0.008$) (Table 4), while species richness index was greater in agriculture streams, but only during spring ($P=0.009$) and fall ($P=0.030$) (Table 4). Shannon–Wiener diversity ($P<0.001$) and species dominance ($P<0.001$) values were both greater in agriculture streams, though only during spring (Table 4). Stream type exhibited no differences with species evenness, Centrarchidae relative abundance, or Poeciliidae relative abundance (Table 4).

Table 2 Relative abundances of fish families collected from agriculture and forest streams of the Mississippi Alluvial Plain

Family	Common name	Relative abundance	
		Agriculture (%)	Forest (%)
Cyprinidae	Minnows	34.4	80.9
Centrarchidae	Sunfishes, crappies	33.2	7.5
Aphredoderidae	Pirate perch	12.3	0.9
Poeciliidae	Live-bearers	6.7	0.7
Ictaluridae	North American catfishes	6.3	0.2
Atherinopsidae	Silversides	0.2	3.7
Fundulidae	Topminnows	0.4	3.4
Percidae	Darters, perches	2.2	1.5
Lepisosteidae	Gars	2.6	0.4
Clupeidae	Shads	0.6	0.8
Catostomidae	Suckers	0.4	< 0.1
Sciaenidae	Freshwater drum	0.3	< 0.1
Elassomatidae	Banded pigmy sunfishes	0.3	< 0.1

Canonical correspondence analysis included 16 of 57 species, each of which comprised > 1% of the total catch. The first two canonical axes of CCA (CA1 and CA2) accounted for 46% of the cumulative variance explained, with the third axis (CA3) explaining an additional 17% (Table 5). The Monte Carlo test was significant ($P=0.002$), indicating that the model's observed eigenvalues were greater than those generated from randomized data, which indicated a significant association between fish assemblages and the environmental variables measured. However, P-values were not calculated for CA2 and CA3 due to the risk of biased values resulting from the Monte Carlo test used (McCune and Grace 2002).

CCA results indicated that fish assemblages in MAP forest and agriculture streams were structured primarily along a gradient that reflected instream habitat conditions, water nutrient concentrations, and benthic chl-*a* production (Table 5, Fig. 6). Secondarily, assemblages were structured along a mostly water quality gradient composed of variables reflecting water ionic strength (e.g., conductance, pH, salinity, and alkalinity)

Table 3 Numbers, relative abundances, trophic guilds, and environmental tolerances of 57 fish species collected in forest ($n = 2237$) and agriculture ($n = 1298$) streams of the Mississippi Alluvial Plain ecoregion

Common Name *Scientific name*	**Trophic guild***	**Tolerance ***	**Agriculture**		**Forest**	
			n	**%**	***n***	**%**
Banded Pygmy Sunfish *Elassosoma zonatum*	I	M	4	0.3	0	0.0
Black Bullhead *Ameiurus melas*	I	M	1	< 0.1	0	0.0
Black Crappie *Pomoxis nigromaculatus*	P	M	9	0.7	15	0.7
Blackside Darter *Percina maculata*	I	M	1	< 0.1	0	0.0
Blackspotted Topminnow *Fundulus olivaceus*	I	M	5	0.4	59	2.6
Blackstripe Topminnow *Fundulus notatus*	I	M	0	0.0	18	0.8
Blacktail Shiner *Cyprinella venusta*	I	M	2	0.2	11	0.5
Bluegill *Lepomis macrochirus*	I	M	151	11.6	109	4.9
Bluntnose Darter *Etheostoma chlorosomum*	I	M	16	1.2	11	0.5
Brook Silverside *Labidesthes sicculus*	I	M	2	0.2	82	3.7
Bullhead Minnow *Pimephales vigilax*	O	M	56	4.3	3	0.1
Channel Catfish *Ictalurus punctatus*	P	M	10	0.8	1	< 0.1
Common Carp *Cyprinus carpio*	O	T	2	0.2	2	< 0.1
Crystal Darter *Crystallaria asprella*	I	I	0	0.0	2	< 0.1
Cypress Darter *Etheostoma proeliare*	I	M	6	0.5	0	0.0
Cypress Minnow *Hybognathus hayi*	O	M	13	1.0	5	0.2
Dusky Darter *Percina sciera*	I	M	1	< 0.1	9	0.4
Emerald Shiner *Notropis atherinoides*	I	M	23	1.8	884	39.5
Flathead Catfish *Pylodictis olivaris*	P	M	7	0.5	1	< 0.1
Freshwater Drum *Aplodinotus grunniens*	V	M	4	0.3	0	0.0
Ghost Shiner *Notropis buchanani*	I	M	21	1.6	6	0.3
Gizzard Shad *Dorosoma cepedianum*	O	M	8	0.6	17	0.8
Golden Shiner *Notemigonus crysoleucas*	O	T	10	0.8	3	0.1
Green Sunfish *Lepomis cyanellus*	I	T	15	1.2	13	0.6
Harlequin Darter *Etheostoma histrio*	I	I	0	0.0	1	< 0.1
Ironcolor shiner *Notropis chalybaeus*	I	I	1	< 0.1	0	0.0
Logperch *Percina caprodes*	I	M	0	0.0	1	< 0.1
Longear sunfish *Lepomis megalotis*	I	M	0	0.0	4	0.2
Longnose Gar *Lepisosteus osseus*	P	M	5	0.4	4	0.2
MimicShiner *Notropis volucellus*	I	I	12	0.9	6	0.3
Mud Darter *Etheostoma asprigene*	I	M	0	0.0	4	0.2
Orangespotted Sunfish *Lepomis humilis*	I	M	135	10.4	6	0.3
Pallid Shiner *Notropis amnis*	I	I	9	0.7	89	4.0
Pirate Perch *Aphredoderus sayanus*	I	M	159	12.3	20	0.9
Pugnose Minnow *Opsopoeodus emiliae*	I	I	33	2.5	18	0.8
Red Shiner *Cyprinella lutrensis*	O	T	146	11.3	1	< 0.1
Redear Sunfish *Lepomis microlophus*	I	M	16	1.2	0	0.0
Ribbon Shiner *Lythrurus fumeus*	I	M	0	0.0	2	< 0.1
River Darter *Percina shumardi*	I	M	2	0.2	2	< 0.1
River Shiner *Notropis blennius*	I	M	90	6.9	185	8.3
Shorthead Redhorse *Moxostoma macrolepidotum*	I	M	0	0.0	1	< 0.1
Shortnose Gar *Lepisosteus platostomus*	P	M	9	0.7	1	< 0.1
Silverband Shiner *Notropis shumardi*	I	M	15	1.2	16	0.7
Slough Darter *Etheostoma gracile*	I	M	2	0.2	1	< 0.1
Smallmouth Buffalo *Ictiobus bubalus*	I	M	5	0.4	0	0.0
Shoal Chub *Macrhybopsis hyostoma*	I	I	1	< 0.1	8	0.4
Spotted Bass *Micropterus punctulatus*	P	M	0	0.0	3	0.1

Table 3 (continued)

Common Name *Scientific name*	Trophic guild*	Tolerance *	Agriculture		Forest	
			n	**%**	***n***	**%**
Spotted Gar *Lepisosteus oculatus*	P	M	20	1.5	4	0.2
Spotted Sunfish *Lepomis punctatus*	I	M	4	0.3	1	< 0.1
Tadpole Madtom *Noturus gyrinus*	I	M	63	4.9	2	< 0.1
Taillight Shiner *Notropis maculatus*	I	M	3	0.2	2	< 0.1
Warmouth *Lepomis gulosus*	P	M	59	4.6	9	0.4
Weed Shiner *Notropis texanus*	I	I	10	0.8	569	25.4
Western Mosquitofish *Gambusia affinis*	I	M	87	6.7	16	0.7
White Bass *Morone chrysops*	P	M	2	0.2	0	0.0
White Crappie *Pomoxis annularis*	P	M	42	3.2	10	0.5
Yellow Bullhead *Ameiurus natalis*	I	T	1	< 0.1	0	0.0

*Trophic guilds: P = piscivore, I = insectivore, O = omnivore, V = generalized invertivore. **Tolerances: I = intolerant, M = moderately tolerant, T = tolerant (Barbour et al. 1999)

Table 4 Repeated-measures ANOVA of fish assemblage (mean ± SD) variables in agriculture and forest streams of the Mississippi Alluvial Plain

Variable	Agriculture	Forest	Season	Main effect (type)	Interaction (season × type)	
Total fish abundance	36 ± 28	62 ± 110	0.014	0.322		**0.041**
Species richness	7 ± 3	5 ± 4	0.097	0.080		0.115
Species richness index	1.48 ± 0.25	1.19 ± 0.44	0.035	0.083		**0.017**
					Spring	**0.009**
					Fall	**0.030**
Shannon–Wiener diversity	1.44 ± 0.34	0.91 ± 0.50	0.276	< 0.001		**0.034**
					Spring	**< 0.001**
Species evenness	0.80 ± 0.13	0.78 ± 0.22	0.445	0.097		0.392
Species dominance	0.68 ± 0.12	0.47 ± 0.24	0.283	< 0.001		**0.026**
					Spring	**< 0.001**
Intolerant species relative abundance	0.06 ± 0.12	0.28 ± 0.19	0.020	0.005		**0.007**
					Spring	**< 0.001**
					Fall	**0.015**
Centrarchidae relative abundance	0.41 ± 0.27	0.16 ± 0.21	0.831	0.119		0.125
Cyprinidae relative abundance	0.28 ± 0.23	0.49 ± 0.24	0.495	**0.047**		0.492
Percidae relative abundance	0.03 ± 0.06	0.14 ± 0.17	0.335	**0.032**		0.495
Poeciliidae relative abundance	0.11 ± 0.14	0.03 ± 0.10	0.162	0.260		0.316

Data were collected in spring, summer, and fall 2014. Significant effects ($P \leq 0.05$) that were interpretable are denoted with bold print

and benthic chl-*a* production (Table 5, Fig. 6). There was clear separation in multivariate space between fish assemblages in agriculture and forest streams along CA1, though the separation was weaker along CA2 (Fig. 6). However, there also was overlap in fish assemblages between the two stream types, which suggested many common species occurred (Fig. 6). In general, fish assemblages in forest streams were associated with greater habitat scores and lower concentrations of benthic chl-*a*, total phosphorus, nitrate, and phosphate (Fig. 6). Conversely, fish assemblages in agriculture streams were associated with greater concentrations of water nutrients and related measures (e.g., benthic chl-*a*) (Fig. 6). There appeared to be little structuring among seasons, meaning that spring, summer, and fall fish assemblages in both stream types contained many common species.

Several fish species did appear to exhibit affinities for a particular stream type. Fish species more associated with agriculture streams in the MAP included bullhead minnow (*Pimephales vigilax*) (PIVI), orangespotted

Table 5 Canonical correspondence analysis of fish species and environmental characteristics for agriculture and forest streams of the Mississippi Alluvial Plain

Axis summary statistics	CA1	CA2	CA3
Eigenvalue	0.762	0.718	0.567
% of variance explained	23.5	22.1	17.5
Cumulative % variance explained	23.5	45.6	63.1
Environmental variables:	***r***	***r***	***r***
Temperature	− 0.256	− 0.185	− 0.085
Dissolved oxygen	0.130	**− 0.331**	− 0.100
Specific conductance	− 0.234	**− 0.549**	0.036
Total suspended solids	− 0.146	0.046	0.279
Chloride	0.033	− 0.222	0.290
Alkalinity	− 0.186	**− 0.515**	**− 0.318**
Total nitrogen	− 0.179	**0.347**	− 0.014
Total phosphorus	**− 0.617**	0.155	0.085
Benthic chlorophyll-*a*	**− 0.421**	**− 0.575**	− 0.231
Water-column chlorophyll-*a*	− 0.250	− 0.235	0.219
Total habitat score	**0.533**	**0.651**	− 0.063
Salinity	− 0.274	**− 0.515**	− 0.010
pH	− 0.098	**− 0.594**	− 0.268
Turbidity	− 0.131	0.016	**0.455**
Total ammonia nitrogen	− 0.222	− 0.047	− 0.108
Nitrate	**− 0.345**	− 0.062	**0.401**
Phosphate	**− 0.573**	− 0.105	0.260
Hardness	− 0.104	0.096	− 0.014

sunfish (LEHU), warmouth (*Lepomis gulosus*) (LEGL), pirate perch (APSA), red shiner (CYLU), white crappie (*Pomoxis annularis*) (POAN), tadpole madtom (*Noturus gyrinus*) (NOGY), and western mosquitofish (GAAF) (Table 3, Fig. 6). Species more associated with MAP forest streams included blackspotted topminnow (*Fundulus olivaceus*) (FUOL), brook silverside (*Labidesthes sicculus*) (LASI), emerald shiner (NOAT), pallid shiner (*Hybopsis amnis*) (HYAM), and weed shiner (NOTE) (Table 3, Fig. 6). Agriculture and forest streams had several species in common, including bluegill (LEMA), pugnose minnow (*Opsopoeodus emiliae*) (OPEM), and river shiner (NOBL) among others. Interestingly, river shiner is actually considered more of a large-river species in the MAP region, being less common in smaller, lower-order systems like those studied (Robison and Buchanan 2020).

The trophic composition and disturbance tolerance of fish assemblages also varied by stream type in the MAP. Forest streams were largely insectivore dominated (97%), with lower relative abundances of omnivores (1%) and piscivores (2%). Conversely, agriculture streams contained greater relative abundances of both omnivores (18%) and piscivores (13%), and lower abundances of insectivores (69%). Both stream types were dominated by moderate-tolerance species (68–81% of the total catches). However, forest streams tended to have greater relative abundances of intolerant species (31% compared to 6% in agriculture streams) while agriculture streams had greater relative abundances of tolerant species (13% compared to < 1% in forest streams).

Discussion

Agriculture appeared to significantly affect the physical attributes of MAP streams at local scales. Total habitat, instream habitat, and riparian habitat scores all were consistently greater in forest streams throughout all three seasons, which suggested that row-crop agriculture likely affected both instream habitat and riparian conditions. Stream-type differences observed with all three habitat measures appeared to be driven by the score components that reflected substrate and pool characteristics, and the quality of stream banks and riparian zones, which all trended towards better scores in forest streams. In agriculture streams, habitat measures appeared to be influenced directly by agriculture occurring in the watersheds, with many of those practices occurring within sight of the receiving streams.

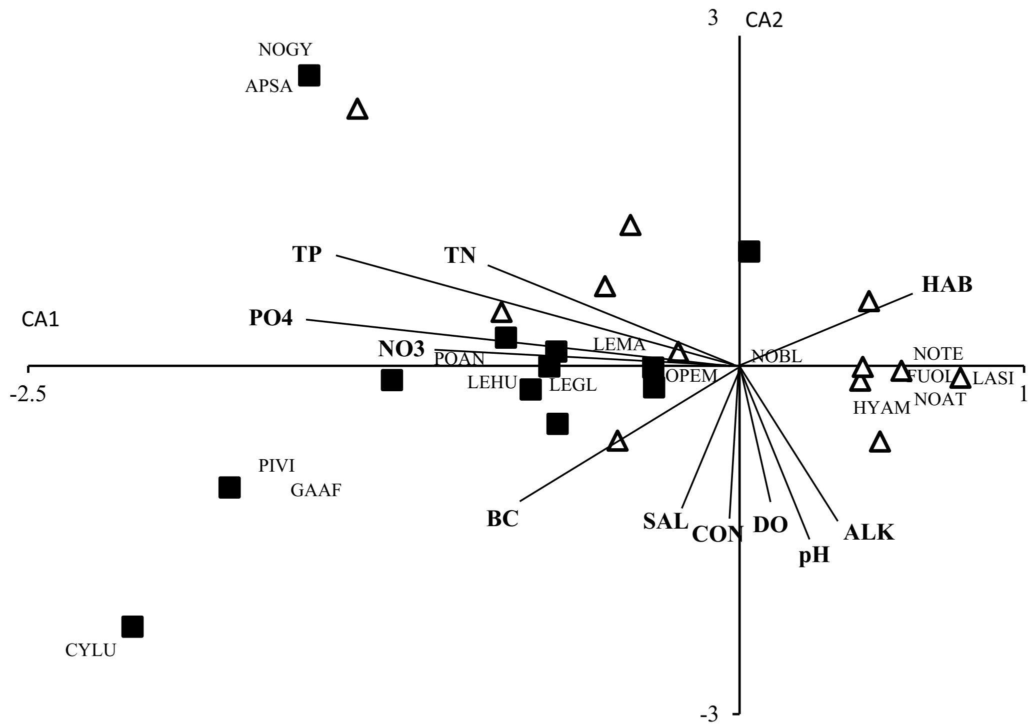

Fig. 6 Scatterplot of the first (CA1) and second (CA2) axes of canonical correspondence analysis of fish assemblage composition and environmental variables in agriculture and forest streams of the Mississippi Alluvial Plain. Triangles represent forest streams; squares represent agriculture streams. Sixteen fish species that comprised > 1% of the total catch each were included in the analysis. Vectors represent environmental variables with variable–axis correlations of either ≤ – 0.3 or ≥ 0.3. Species codes: LEGL = warmouth; LEHU = orangespotted sunfish; LEMA = bluegill; POAN = white crappie; OPEM = pugnose minnow; HYAM = pallid shiner; NOTE = weed shiner; CYLU = red shiner; PIVI = bullhead minnow; NOAT = emerald shiner; NOBL = river shiner; NOGY = tadpole madtom; GAAF = western mosquitofish; APSA = pirate perch; LASI = brook silverside; FUOL = blackspotted topminnow. Environmental variable codes: DO = dissolved oxygen; CON = specific conductance; ALK = alkalinity; TN = total nitrogen; TP = total phosphorus; BC = benthic chlorophyll-*a*; HAB = total habitat score; SAL = salinity; pH = pH; NO3 = nitrate; PO4 = phosphate

Decreases in habitat condition in streams draining agricultural watersheds are commonly observed (e.g., Roth et al. 1996; Allan 2004; Smiley et al. 2009; Smiley and Gillespie 2010; Riseng et al. 2011; Sanders et al. 2020). Effects are often due to channel straightening and the associated destruction of riparian and instream habitats (Smiley et al. 2009; Sanders et al. 2020). Decreases in habitat condition in MAP streams due to agriculture have been observed previously (e.g., Justus 2003; Wentz et al. 2011; McCarthy et al. 2012), with some streams experiencing large habitat impacts due to irrigation-related water withdrawals (e.g., Yasarer et al. 2020). In general, Wang et al. (1997) suggested that agriculture-related impacts with stream habitats were most detectable when greater than 50% of the watershed contained agricultural activities. MAP streams influenced by agriculture in this study were consistent with this criterion, averaging more than 60% agricultural land cover in 2011.

Similar to physical habitat, agriculture significantly affected water quality in MAP streams, at least at local scales. Agriculture appeared to significantly affect the primary nutrient constituents (i.e., nitrogen and phosphorus) irrespective of season (i.e., season × stream-type interaction was not significant), with mean concentrations consistently between twofold and sixfold greater in agriculture streams. Middle-order streams in the MAP tend to be turbid and nutrient-rich regardless, though agricultural activities (e.g., row crops) undoubtedly exacerbate the nutrient and turbidity issues. Regardless of season, agriculture streams in this study consistently contained greater levels of nitrate, phosphate, total nitrogen, and total phosphorus compared to forest streams. Similarly, TAN concentrations were significantly greater in agriculture streams during summer coincident with lower stream flows. Increased instream concentrations of these particular nutrient measures (i.e., nitrate, TAN, total nitrogen, phosphate, and total phosphorus) have been associated with agriculture in previous studies. Nutrient levels in these streams are largely resultant from overland runoff in concert with widespread use of inorganic fertilizers (Jordan et al. 1997; Rohm et al. 2002; Riseng et al. 2011).

Increased nutrients (especially phosphorus) have been documented previously in MAP streams with highly agricultural watersheds (Rohm et al. 2002; Wentz et al. 2011; Shrestha et al. 2017). Nitrogen levels in MAP streams are usually not excessive, though they tend to be highly variable (e.g., Giese et al. 1987; Stephens et al 2008; Wentz et al. 2011; Shrestha et al. 2017). Previous studies in many MAP streams also reported highly variable nitrogen concentrations, though levels in this study were less variable such that significantly greater nitrogen concentrations were consistently detected in agriculture streams.

Agriculture effects on turbidity and TSS in MAP streams were confounded by season. Turbidity and TSS levels were consistently greater in agriculture streams, though differences were limited to higher-flow periods common during spring. This observation was undoubtedly due to elevated seasonal runoff from spring rains in conjunction with row-crop fields being essentially barren during that time of year. Increases in TSS and turbidity are not uncommon in streams draining agricultural areas. Furthermore, high variation in TSS and turbidity as observed in this study also have been reported in previous studies of MAP streams (Stephens et al. 2008; Wentz et al. 2011; Shields et al. 2013; Shrestha et al. 2017). The lack of historical data from agriculture streams used in this study precludes precise estimation of total sediment loads, and thus, determination of the proportion of sediment runoff attributable to agriculture was not possible. In any event, generally decreased water quality conditions in streams with highly agricultural watersheds is common in many areas of the world (Allan 2004; Schűrings et al. 2022).

Fish assemblages in agriculture and forest streams of the MAP exhibited distinct structural differences. The lower values for diversity, evenness, and dominance in forest streams were unexpected, but explainable in that the 72% greater fish abundances in forest streams on average did not include significant numbers of additional species that were not found in agriculture streams. However, three common cyprinids found in agriculture streams (emerald shiner, weed shiner, and pallid shiner) were 37-fold more abundant in forest streams. Thus, the greater overall fish abundances in forest streams coupled with the substantially greater abundances of these three species would result in depressed diversity, evenness, and dominance values in forest streams, though we would not conclude that fish assemblages forest streams were unhealthy or imbalanced. This general finding was similar to Shrestha et al. (2017) from smaller, headwater MAP streams whereby differences in fish assemblages could not be detected from streams with row-crop dominated watersheds compared to streams draining watersheds containing constructed wetlands that had been row-crop dominated years before.

Multivariate analyses did indicate structural differences between agriculture and forest streams that were not evident from richness, diversity, evenness, and dominance analyses. CCA indicated that MAP fish assemblage compositions were largely structured along gradients that reflected instream habitat conditions, water nutrient concentrations, and levels of benthic chl-*a*. All of these variables consistently differed between MAP agriculture and forest streams despite that study streams were distributed across multiple river basins. However, results further suggested that agriculture impacts affected fish assemblage functional composition in MAP streams. The relative abundance of omnivores in fish assemblages is a common measure reflecting assemblage health, with greater abundances of omnivores indicative of environmental stress, which often leads to trophic imbalance (Karr 1981; Fausch et al. 1984). In this study, relative abundances of omnivores in agriculture streams (mainly red shiners and bullhead minnows) were 13-fold greater than forest streams. Similarly, the relative abundances of insectivores (largely cyprinids) were 40% greater on average in forest streams compared to agriculture streams. High relative abundances and diversity of insectivores is often associated with healthy fish assemblages (Karr 1981; Fausch et al. 1984). Although 40 of the 57 species collected in this study were classified as insectivores following Barbour et al. (1999), the greater relative abundance of insectivores in forest streams coupled with the greater total abundances of all fishes in forest streams (2237 compared to 1298 in agriculture streams) is likely related to the significantly better habitat conditions detected in forest streams. With lower TSS and turbidity values, forest streams in this study contained more gravel-cobble substrates that were cleaner and less embedded than agriculture streams, which would translate to much greater abundances of aquatic macroinvertebrates. This characteristic would provide optimal habitats for most of the insectivores collected (Robison and Buchanan 2020), nearly all of which were cyprinids and percids. Conversely, streams with more degraded instream habitats that contained siltier substrates would likely be more conducive to omnivory, which was observed to some extent in our agriculture streams. Land use changes, including agriculture, affecting the functional composition of stream fish assemblages has been reported in previous studies (e.g., Justus 2003; Alvarenga et al. 2021; Hughes and Vadas 2021; Schűrings et al. 2022).

Another functional attribute that agriculture appeared to have affected in MAP streams was the relative environmental tolerance of the fish assemblages. In our context, "environmental tolerance" reflected the general tolerance of the fish species to non-specific environmental stressors following Barbour et al. (1995, 1999). These non-specific stressors represented a broad spectrum of environmental stressors, including organic pollution, sedimentation, habitat degradation, and combinations of these stressors (Barbour et al. 1995, 1999). In this study of MAP streams, relative abundances of intolerant fish species were sixfold greater in forest streams than agriculture streams. By comparison, relative abundances of tolerant fish species were 13-fold greater in agriculture streams compared to forest streams. Thus, it was probable that the combination of more degraded habitats, greater nutrient concentrations, and higher turbidities detected in agriculture streams created conditions favoring more tolerant fish species (e.g., red shiners) that could survive a wider array of environmental conditions. The opposite of these conditions in most forest streams allowed more intolerant species to survive, though the vast majority of intolerant specimens in forest streams were weed shiners. Overall, although fish assemblages in agriculture and forest were not greatly different structurally, they did exhibit functional differences that suggested healthier assemblages generally existed in MAP forest streams.

An additional consideration concerning fish assemblage environmental tolerances would be the number of Percidae (i.e., "darter") species collected in MAP streams (sensu Karr 1981). Many percid species are highly sensitive to environmental perturbations and generally indicative of good environmental conditions (Pfleiger 1997; Robison and Buchanan 2020). Overall, 10 percid species were collected from MAP streams, though none were abundant (< 2% of the total catch overall). In particular, eight species collected from forest streams compared to six species from agriculture streams (Table 3). However, of the percid species collected in this study, only three specimens from two species classified as intolerant (one harlequin darter *Etheostoma histrio* and two crystal darters *Crystallaria asprella*) were ever collected, with all being collected from forest streams. Although there could have been gear selectivity issues with regard to the effectiveness of mini-fyke nets and trap nets to sample percids, it may also be that percid species native to the MAP may only have been marginal indicators of instream conditions. This idea would somewhat contradict the fish-based index of biotic integrity (Karr 1981; Fausch et al. 1984) that postulates large numbers of intolerant species (which includes many percids) are indicative of healthy streams. However, key metrics for the index of biotic integrity were not developed from low-gradient, silt-dominated streams with high degrees of agricultural influence such as those found in the MAP. It is likely that an index of biotic integrity suitable for MAP streams might emphasize function (e.g., trophic guilds and environmental tolerances) more so than structure (e.g., percid or centrarchid abundance).

Alterations of the physical and chemical features of streams due to agricultural land use have been observed to induce large, detectable changes in fish assemblage composition (e.g., Lammert and Allan 1999; Waite and Carpenter 2000; Sullivan et al. 2004; Duehr et al. 2006; Alvarenga et al. 2021; Schűrings et al. 2022). Chemical and physical characteristics related to agriculture coupled with local factors (e.g., riparian conditions at the sampling location) may serve as ecological filters that dictate the composition of fish assemblages in receiving streams (Tonn 1990; Poff 1997; Mims and Olden 2013). This phenomenon can result in increased abundance of tolerant species and decreased abundance of sensitive species in streams greatly affected by agriculture (Hrodey et al. 2009). While landscape-level effects have been observed as important in many individual studies (e.g., Alvarenga et al 2021; Xiong et al. 2021), these effects are sometimes diminished when compared to local or within-stream factors (e.g., Marsh-Matthews and Matthews 2000). Nonetheless, land use effects inevitably become detectable as disturbance levels (e.g., agriculture) increase beyond some threshold (Wang et al. 2006). The pervasive effects of agriculture on the chemical and physical characteristics of middle-order MAP streams have likely affected these ecological filters, and thus, influenced the individual species and assemblage compositions occurring in MAP streams.

Conclusions

Although this study considered only point samples over a single year, the results of this study were generally similar to other studies conducted in agricultural streams in the MAP and elsewhere. Generally, it is not debatable that agriculture has altered the physical and chemical conditions (e.g., Justus 2003; Wentz et al. 2011; McCarthy et al. 2012) in middle-order MAP streams to the extent that the resulting fish assemblage is a function of the local conditions at or adjacent to a particular site (e.g., Keith and Shirley 1985; Killgore and Baker 1996; Stephens et al. 2008; Shields et al. 2013; Yasarer et al. 2020). As observed in this study, land use affects the local conditions at a given location, and thus, alters fish assemblage composition in an explainable manner. Row-crop agriculture across the landscape normally results in degraded instream habitats and increased benthic chl-*a,* nutrient loading, and sediment loads resulting in predictable shifts in fish assemblage composition. In these particular MAP streams, the shifts tended to be more functional than structural, and included increased abundances of tolerant species, increased abundances of omnivores, and decreased abundance of insectivores. The results from this study add to the growing body of literature examining differences observed between agriculture streams and appropriate reference streams with respect to physical, chemical, and biological characteristics. In addition, this particular study focused on medium-sized streams, which complements previous work done in the MAP and elsewhere that emphasized headwater systems. The combined results of these studies highlight a common theme such that protecting instream and riparian habitats is critical for various fish species regardless of stream size. Furthermore, protection of forested streams in the MAP from future anthropogenic disturbances using mechanisms such as the U.S. Fish and Wildlife Service's National Wildlife Refuge system is essential towards reducing continued declines in fish biodiversity in areas of rich and/or unique fish diversity (e.g., Indian Bayou; Buchanan 1997), which are generally rare in the MAP during the modern era.

Appendix A: Protocol for selection of alternate sites

Rationale

A common characteristic of MAP streams is that they often become impassable by boat during base flow periods in late summer and fall. This characteristic is due mostly to the normal decreases in seasonal discharge, which is due both to lack of rainfall and intensive agricultural irrigation practices occurring at that time of the year. Thus, alternative sampling locations are sometimes needed to continue stream assessments during drier periods. In this study, 12 sites across six study streams became impassable by boat during fall, and thus, needed alternate sites selected in order to continue sampling. Although headwater streams (i.e., first-order and second-order) emphasized in many previous studies also undergo periods of low discharge, they are often numerically more abundant in stream basin networks to the point that alternate sites in close proximity to the original sampling locations can be easily selected. In a typical river basin, there could be tenfold or more headwater systems compared to larger, more downstream third-order and fourth-order systems (Hauer and Lambert 2007).

During normal sampling in these streams, sites were accessed by hand-transporting a small boat down a stream bank (usually at a bridge or culvert near the sampling site). These boats would be loaded with the necessary sampling equipment. Streams became impassable when water levels became so low that boat movement was restricted. In effect, the stream might be accessible at a bridge or culvert, but the boat could not move upstream or downstream any significant distance due to shallow water. Neither hand-carrying of equipment or portaging of boats was practical in these streams. When an alternate site was needed, these sites were located as near as possible to the original sampling site, though this was largely dictated by the proximity of roads and other crossings. Additionally, private property issues were sometimes an issue such that fencing, no trespassing signs, and locked gates limited our ability to access suitable alternative sites when they were found. This protocol would sometimes result in multiple sites being located in the same location. In these instances, alternate sites had to be relocated to locations further away from original sampling locations. However, we feel this adjustment of site locations did not significantly affect our results. In our repeated-measures ANOVA analyses, "stream" was treated as the experimental unit during all analyses, with individual sites within streams treated as subsamples. Our judgment was that because multiple sampling sites within streams were ultimately being combined into a single experimental unit for analyses (i.e., stream), the potential effects associated with periodic selection of alternate sites would be appropriately buffered and rendered negligible.

Assessment

Following the selection of alternative sites during fall 2014, there were no distinct patterns in the sites relocated relative to agriculture or forest streams nor any trend as to whether sites were relocated in upstream or downstream directions. Alternate sampling sites were chosen for six sites across three forest streams and six sites across four agriculture streams. Similarly, relocated sites within streams were composed of three upstream, three middle, and six downstream sites. Overall, six of the alternate sites were upstream of the original sites and six were downstream. Although some of the alternate sites were up to 7 km from the original sites, most were less than 5 km, though distances tended to be greater at forest sites due to limited road access in parts of the federally managed White River National Wildlife Refuge. When examining average distances between alternate sites and original sites in terms of the standard "40 × stream width" sampling reach definition (Barbour et al. 1999), alternative sites in all streams were on average less than four standard stream reach lengths from the original sites. Given the access and logistical issues that existed in these streams, we did not feel there was any alternative protocol that would have improved our selection of alternate sites.

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Author contributions
YC and MS designed the study, and collected and analyzed the data. MS wrote the original manuscript, with all authors contributing towards finalizing the manuscript.

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Availability of data and materials
All data are available Aquaculture/Fisheries Center at the University of Arkansas at Pine Bluff (UAPB).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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