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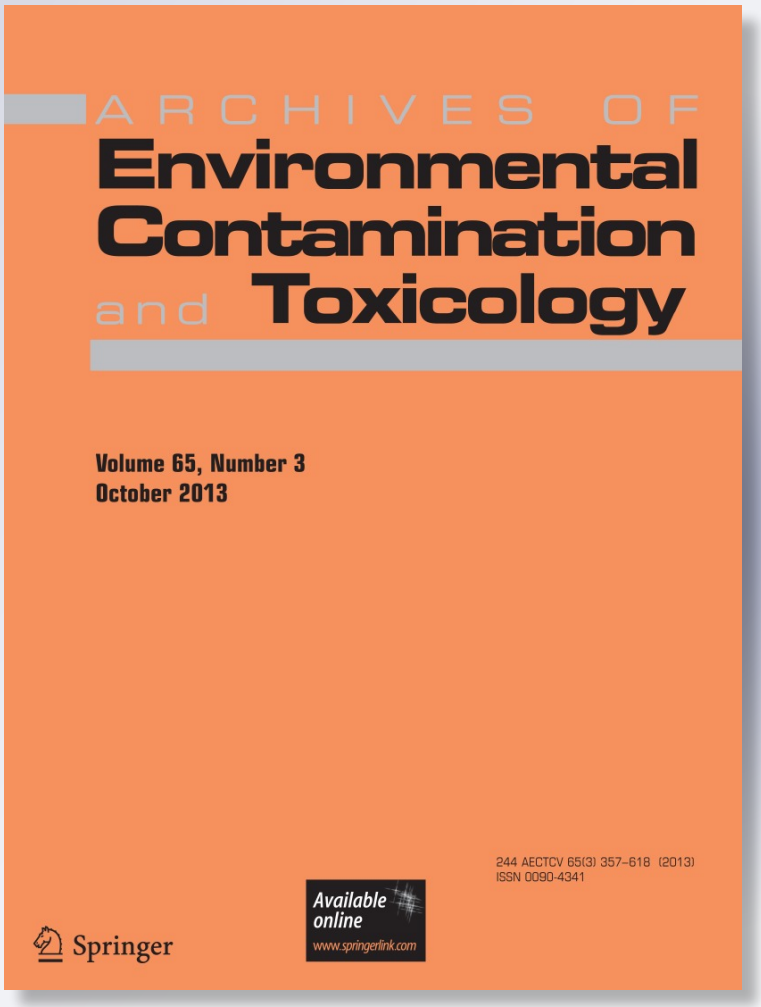

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Responses of Phytoplankton and *Hyalella azteca* to Agrichemical Mixtures in a Constructed Wetland Mesocosm

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Abstract We assessed the capability of a constructed wetland to mitigate toxicity of a variety of possible mixtures, such as nutrients only (NO) (nitrogen [N], phosphorus [P]), pesticides only (PO) (atrazine, *S*-metolachlor, permethrin), and nutrients + pesticides on phytoplankton chlorophyll-*a*, on 48-h aqueous *Hyalella azteca* survival and 10-day sediment *H. azteca* survival and growth. Water and sediment were collected at 10-, 20-, and 40-m distances from inflow and analyzed for nutrients, pesticides, chlorophyll-*a*, and *H. azteca* laboratory bioassays. Phytoplankton chlorophyll-*a* increased 4- to 10 -fold at 7 days after NO treatment. However, responses of chlorophyll-*a* to PO and nutrients + pesticides were more complex with associated decreases at only 20 m for pesticides only and 10 and 40 m for nutrients + pesticides treatments. *H. azteca* aqueous survival decreased within the first 48 h of dosing at 10- and 20-m distances during PO and nutrients + pesticides treatments in association with permethrin concentrations. *H. azteca* sediment survival was unaffected, whereas 10-day growth decreased within 1 day of dosing at all sites during nutrients + pesticides treatment. Constructed wetlands were shown to be an effective agricultural best-management tool for trapping pollutants and mitigating ecological impacts of run-off in agricultural watersheds.

In the past century, agriculture has been critical for providing food and fiber in sustaining an ever-growing human population, and projected population increases are expected to place greater demands on agricultural productivity and sustainability (Tilman et al. 2011). As a result, the use of fertilizers and pesticides to maximize yields has increased concomitantly (Robertson and Vitousek 2009) leading to potential ecological impacts to aquatic biota in agricultural watersheds (Phillips et al. 2006). Agricultural best-management practices (BMPs) targeting mitigation of nutrients and pesticides in agricultural run-off are increasing being used, and one such BMP is the vegetated surface flow constructed wetland (Braskerud 2002; Budd et al. 2009; García-Lledó et al. 2011; Díaz et al. 2012). Constructed wetlands used in agricultural watersheds have important economic and ecological functions, including acting as filters and processors of a variety of agricultural contaminants, such as suspended sediment, nutrients, and pesticides entering from adjacent agricultural fields during run-off events (Johnson 1986; Cairns 2007) even if such constructed ecosystems are functionally more limited than natural systems (Moreno-Mateos et al. 2012). Despite these limitations, constructed wetland systems provide diverse habitats and refuge for a high diversity of wildlife and aquatic biota, such as communities of phytoplankton and aquatic invertebrates (Detenbeck et al. 1996; Cairns 2007).

The use of aquatic mesocosms to assess the functional processes and responses of a natural aquatic system to stressors has been used to provide realistic exposure–response conditions observed under field conditions (Wendt-

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Rasch et al. 2004; Lizotte et al. 2011). As a result, wetland mesocosms are an essential tool in addressing issues of ecosystem responses of constructed wetland habitats used to intercept agricultural run-off. Because such run-off frequently occurs as a variety of complex mixtures, including nutrients, pesticides, and combinations of both (Anderson et al. 2003; Budd et al. 2009; Díaz et al. 2012), an understanding of the ecological effects of these mixtures is necessary to develop optimal constructed wetland specifications to maximize decreases in pollutants and concomitant improvements in ecological integrity both within the wetland habitat and to downstream receiving water bodies.

The objectives of the current study were to examine the capability of a constructed wetland to mitigate the toxicity of three of potential agrichemical mixtures, including nutrients-only (NO) (nitrogen [N], phosphorus [P]), pesticides-only (PO) (atrazine, *S*-metolachlor, permethrin), and nutrients + pesticides (N + P) to water column phytoplankton (measured as chlorophyll-*a*) and a sentinel epibenthic crustacean (*Hyalella azteca*) using both aqueous and sediment phases.

Materials and Methods

Experimental Design

The 455 m^2 constructed wetland mesocosm used in the current study was a naturally vegetated free water surface wetland located at the University of Mississippi Field Station (UMFS) (34°25′55″ N, 89°24′00″ W) in Lafayette County, MS, USA. Dominant vegetation included common rush (*Juncus effusus*) and common reed (*Phragmites australis*) with occasional rice cutgrass (*Leersia oryzoides*) and swamp maple (*Acer rubrum*). The wetland was split into two latitudinal sections (approximately 227 m^2 each) and hydraulically isolated along its longitudinal axis with a 6-mil plastic liner placed 0.3 m lower than the sediment surface and standing 1.2 m above the wetland bed. The study included a control condition [no amended contaminants (CO)] and simulated three different agricultural run-off event scenarios with differing contaminant mixture inputs: NO (N and P), PO (two herbicides and one insecticide), and N + P. Run-off volume was based on a 2.5-mm rainfall event off of a 0.9-ha agricultural field generating a total inflow volume of 24,777 L of run-off in each section. The first treatment exposure occurred from July 29 to August 26, 2009, where the first section was amended during a 4-h period on July 29, 2009, with an NO aqueous mixture of ammonium nitrate (825 g N) and triple superphosphate (825 g P), and the second section was amended during the same period with only spring water (CO). After the first treatment but before the start of the second treatment, the wetland was drained and flushed with fresh unamended spring water for 48 h. The second treatment occurred from September 2 to 30, 2009, where the first section was amended during a 4-h period on September 2, 2009, with a PO aqueous mixture of atrazine [8.2 g active ingredient (a.i.)], *S*-metolachlor (6.3 g a.i.), and permethrin (1.33 g a.i.), whereas the second section was amended during the same period with a N + P aqueous mixture of ammonium nitrate (357 g N), super triple phosphate (409 g P), atrazine (8.2 g a.i.), *S*-metolachlor (6.3 g a.i.), and permethrin (1.33 g a.i.).

Although the total experimental exposure period occurred during a 9-week period, including the first week after the autumnal equinox, seasonal effects of light level and temperature differences were mitigated by minimizing the time periods between the first and second exposure periods while efficiently maximizing the observation periods (4 weeks). Temperature was measured continuously every hour in both wetland cells during both treatment exposures. Average weekly temperatures ranged from 22.4 to 27.3 °C during the entire 9-week period (Supplemental Table 1) indicating minimal differences in average measured weekly temperatures between the two exposure periods. Target nutrient and pesticide concentrations were based on previously reported results of these contaminants in row-crop agricultural run-off (Moorman et al. 2004; Southwick et al. 2003). Target pesticides were selected because of their widespread use on one of the largest row-crops, i.e., corn, in the United States to control weed and insect pests. Approximately 23.2 mkg of atrazine, 9.9 mkg of *S*-metolachlor, and 33,000 kg of permethrin were applied to corn crops in the United States in 2010 (the most recent year with complete data) (National Agricultural Statistical Service 2013).

Sample Collection and Analyses

Water

During all treatments, water (1–2 L) was collected every 30 min within the first 4 h; every 4 h until 48 h; and on days 5, 7, 14, 21, and 28 after amendment at distances of 10, 20, and 40 m from the point of amendment in both sections of the wetland. Samples were preserved on wet ice and transported to the United States Department of Agriculture-Agricultural Research Service National Sedimentation Laboratory, Oxford, MS, USA, for chemical and biological analyses. Each sample was analyzed for dissolved inorganic N (DIN; NH_4^+, NO_3^-, and NO_2^-), total N (TN; sum of total kjeldahl N + NO_3^- + NO_2^-), soluble-reactive P (SRP), total P (TP), and dissolved organic carbon (DOC) according to standard methods (APHA 2005; Lizotte et al. 2011). Water samples for DIN, SRP, and

DOC were filtered using a 0.45-μm cellulose nitrate filter before analysis. Phytoplankton photosynthetic pigment chlorophyll-*a* was measured using a pigment extraction and spectrophotometric determination method (trichromatic method) as described by APHA (2005).

Sediment

Surface bulk sediment (upper 5 cm) samples were collected for PO and N + P treatment amendment 6 days prior to agrichemical amendment and days 1, 5, 12, and 22 at distances of 10, 20, and 40 m from the point of amendment. Samples were collected using a stainless steel trowel to transfer 1.9 kg of sediment into 1-L amber-colored glass jars fitted with Teflon-lined screw caps. Sediment sample jars were preserved and transported as per water samples as previously described. Each sediment sample was thoroughly homogenized, and an aliquot (approximately 200 g w/w) was subsampled, air dried for ≤48 h, and finely ground for characterization and nutrient and pesticide analysis. Sediment particle size distribution (sand, silt, clay) was determined according to methods described by Schaff et al. (2003). Additional sediment sample fractions (approximately 1 g) were analyzed for total organic carbon (TOC) and TN according to methods described by Lizotte et al. (2011).

Water and sediment samples for pesticide analysis were assessed using methods modified from Smith et al. (2007) and Bennett et al. (2000). Briefly, target pesticides were extracted from their matrix (water or sediment) by way of pesticide-grade ethyl acetate. Next, samples were concentrated to near dryness by rotary evaporation with anhydrous Na_2SO_4 followed by silica gel column gas chromatography cleanup. The extract was then concentrated to 1-mL volume under high-purity dry N for analysis. An Agilent Model 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany)—equipped with dual Agilent 7683B series autoinjectors, dual split-splitless inlets, dual capillary columns, an Agilent ChemStation, and with the autoinjector set at 1.0-μL injection volume—were used for atrazine, *S*-metolachlor, and permethrin analyses. Carrier gas used was ultra-high purity (UHP) helium at 28 mL/min and the inlet temperature at 250 °C. The microelectron capture detector temperature was 325 °C with a constant make-up gas flow of 60 mL/min UHP N. Detection limits for aqueous samples were 0.1 μg L^{-1} for all pesticides. Detection limits for dry sediment samples were 1 ng g^{-1} for all pesticides.

Crustacean Bioassays

For whole-effluent aqueous animal bioassays, 1-L water samples (hardness adjusted to approximately 100 mg $CaCO_3$/L using $CaCl_2$ and $NaHCO_3$ due to the soft nature of UMFS water) were collected 1 day before PO and N + P treatment amendment and again 4 h, 1, 2, 5, and 7 days after amendment at distances of 10, 20, and 40 m from the point of amendment. Aqueous bioassays were performed in a Powers Scientific incubator (Powers Scientific, Pipersville, Pennsylvania, USA) at 23 ± 1 °C with a 16:8-h light-to-dark photoperiod. Aqueous wetland bioassays were 48-h static nonrenewed exposures measuring *H. azteca* survival within five serial dilutions (at 0.25 dilution factor) of four replicates each according to modified USEPA (2000, 2002) protocols for *H. azteca* reference toxicity tests. *H. azteca* passing through a 600-μm stainless steel mesh sieve, but retained by a 425-μm stainless steel mesh sieve (approximately 1–2 weeks old), were collected for the bioassays. Five *H. azteca* were placed in each 88-mL polypropylene plastic replicate test chamber with one 2 cm × 2 cm square sterile cotton gauze as substrate and 70 mL sample and/or CO/dilution water. Control and dilution water, free from priority pollutants, were from a naturally spring-fed pond located at UMFS with the following ranges of measured water-quality parameters: dissolved oxygen (mg L^{-1}) 4.5–12.6; pH, 5.9–7.3; alkalinity (mg L^{-1} as $CaCO_3$) 8–16; hardness (mg L^{-1} as $CaCO_3$) 10–30; conductivity (μS cm^{-1}) 20.3–25.7; turbidity 8.3–25.1; TP (μg L^{-1}) 0–81; NH_4^+ (μg L^{-1}) 0–136; NO_3^- (μg L^{-1}) 42–170; NO_2^- (μg L^{-1}) 1–15; and chlorophyll-*a* (μg L^{-1}) 0–23.

Bulk sediment animal bioassays were 10-day static nonrenewed exposures assessing *H. azteca* survival according to modified protocols from USEPA (2000) for *H. azteca* survival and growth tests for sediments. *H. azteca* were collected as described for aqueous bioassays. Exposure chambers were 240-mL polypropylene plastic beakers containing 20 g wet weight of homogenized whole bulk sediment and 180 mL of overlying UMFS CO water. Ten *H. azteca* were placed in each of four replicate exposure chambers with two 6 mm-diameter maple leaf discs as substrate and supplemental nutrients. Sediment bioassays were incubated as described for aqueous bioassays. Control sediment and overlying water, free from priority pollutants, were from a naturally spring-fed pond located at UMFS. Physicochemical characteristics measured in overlying water were temperature, dissolved oxygen, pH, alkalinity, hardness, conductivity, nitrite, nitrate, and ammonium using standard methods (APHA 2005).

Statistical Analyses of Data

Phytoplankton chlorophyll-*a* data were analyzed using forward stepwise linear regressions according to Berenson et al. (1983) to assess multiple independent variables that could influence observed changes in dependent variable chlorophyll-*a* concentrations. Data were $\log_{10}$-transformed when appropriate for chlorophyll-*a* (dependent variable) and independent variables of amended agrichemicals

[N + P (Table 1)] to generate the best predictive models during each treatment at distances of 10, 20, and 40 m from amendment inflow during the 28-day postamendment period to examine spatial and temporal changes in concentration-responses using SigmaPlot v. 12.0 statistical software (SYSTAT 2011). To account for the potential confounding influence of rainfall during the temporally separate treatment exposures, the precipitation-independent variable was forced into the equation. Remaining independent variables were then added or removed using F-to-enter of 4.0 and F-to-remove of 3.9 to maximize the robustness of the models and minimize type I and type II errors (SYSTAT 2011). Standardized dimensionless regression coefficients and coefficients of determination were calculated and reported for each variable.

Aqueous *H. azteca* 48-h survival data were arcsin-square root transformed to conform to parametric assumptions before analysis (Berenson et al. 1983). Point estimation methods to estimate 10 % (LC_{10}) and 50 % (LC_{50}) lethal effluent dilution fractions (%) and their 95 % confidence intervals (CIs) were calculated using probit, logit, angular, or weibull analytical methods, when appropriate (Kerr and Meador 1996; Carroll 2003; Fairchild et al. 2005), with ToxCalc v. 5.0.32 toxicity data analysis software (ToxCalc 2008). Acute toxic units (TUs) were determined using methods described by Pape-Lindstrom and Lydy (1997) for nutrient and pesticide components of the mixture in PO and N + P treatments. Aqueous *H. azteca* survival TUs for NH_4^+ 39.8 mg L^{-1} (Ankley et al. 1995), NO_2^- 12.5 mg L^{-1}, NO_3^- 667 mg L^{-1} (Soucek and Dickinson 2012), atrazine 1,500 μg L^{-1} (Ralston-Hooper et al. 2009), metolachlor 6,000 μg L^{-1} (Wan et al. 2006), and permethrin 0.037 μg L^{-1} (Wheelock et al. 2005) were calculated. Nonlinear sigmoidal or logistic regressions were performed, when appropriate, on TUs versus aqueous *H. azteca* survival to ascertain likeliest sources of observed toxicity using SigmaPlot v. 12.0 statistical software (SYSTAT 2011). Bulk sediment *H. azteca* 10-day survival data were log_{10}-transformed, when necessary, to meet parametric assumptions before analysis (Berenson et al. 1983; Schulz et al. 2003). Survival and growth data were analyzed using one-way analysis of variance with Dunnett's multiple range test versus CO when appropriate. Statistical analyses were performed using SigmaPlot v. 12.0 statistical software.

Results

Nutrient and Pesticide Concentrations

Amended aqueous agrichemical concentrations typically peaked within 10 to 24 h after dosing and dissipating to near or lower than preamendment concentrations after

Table 1 Forward stepwise regressions ($n = 26$) computed using chlorophyll-*a* as the dependent variable and varying agrichemical mixtures as independent variables

Treatment	Distance (m)	Standardized dimensionless regression coefficients									
		SRP	DIN	TP	TN	DOC	Atrazine	*S*- metolachlor	Rain	R^2	*p*-value
CO	10[a]		−0.703		**1.017**		N/A	N/A	−0.094	0.500	0.001
CO	20				**0.899**		N/A	N/A	−0.081	0.837	<0.001
CO	40[a]				**1.186**		N/A	N/A	−0.163	0.456	<0.001
NO	10[a]	**−1.156**		0.807			N/A	N/A	0.151	0.559	<0.001
NO	20[b]		**−0.581**			0.431	N/A	N/A	−0.052	0.499	0.001
NO	40					**0.513**	N/A	N/A	0.081	0.244	0.040
PO	10[c]			**0.571**	0.489	−0.340			0.032	0.820	<0.001
PO	20						−2.872	**3.417**	0.209	0.502	0.001
PO	40[a]		−0.667		**1.357**			0.388	0.155	0.754	<0.001
N + P	10[c]	1.036						**−1.725**	−0.150	0.696	<0.001
N + P	20[b]			**−1.557**			0.862		−0.059	0.636	<0.001
N + P	40[c]		−0.481		0.413			−0.296	**0.511**	0.731	<0.001

Standardized dimensionless regression coefficients and coefficients of determination were computed. For each regression, bold font indicates the greatest standardized coefficient and blank cells indicate variables were excluded due to lack of significance. Independent variable "rain" was forced into each equation

CO control, *NO* nutrients only, *PO* pesticides only, *N + P* nutrients + pesticides, *N/A*, not applicable

[a] Log_{10}-transformed dependent and independent variables

[b] Log_{10}-transformed dependent variable

[c] Log_{10}-transformed independent variables

28 days. During CO and NO treatment, peak aqueous DIN concentrations ranged from 2.17 to 45.05 mg L^{-1}, respectively, with greatest concentrations occurring downstream at 40 m for NO (Fig. 1). During PO and N + P treatment, peak aqueous DIN concentrations ranged from 0.82 to 34.23 mg L^{-1}, respectively, with greatest concentrations occurring upstream at 10 m for N + P (Fig. 2). Peak TN aqueous concentrations ranged from 3.83 to 116.91 mg L^{-1} for CO and NO treatment, respectively, with spatial distribution patterns the same as for DIN. PO and N + P treatment peak aqueous TN concentrations ranged from 7.84 to 79.53 mg L^{-1}, respectively, and were spatially distributed in conjunction with DIN. Similar patterns occurred for P where CO and NO treatment peak aqueous concentrations of SRP ranged from 0.99 to 6.87 mg L^{-1}, respectively, with greatest concentrations occurring at 40 m for NO (Fig. 1). PO and N + P treatments showed peak aqueous concentrations of SRP ranging from 0.28 to 7.24 mg L^{-1}, respectively, with greatest concentrations occurring at 10 m for N + P (Fig. 2). Total P peak concentrations during CO and NO treatment ranged from 0.12 to 1.17 mg L^{-1}, respectively, with the greatest concentrations occurring at 40 m for NO. Peak TP concentrations during PO and N + P ranged from 0.13 to 8.47 mg L^{-1}, respectively, with similar spatial patterns of distribution as SRP.

Aqueous pesticide concentrations before amendment were all lower than detection limits of 1 μg L^{-1} for both PO and N + P treatments. Herbicides, atrazine, and *S*-metolachlor showed similar peak concentrations during both treatments ranging from 192 to 197 μg L^{-1} and 288 to 316 μg L^{-1}, respectively (Fig. 2). The insecticide permethrin had peak concentrations ranging from 14 to 23 μg L^{-1} during PO and N + P treatments (Fig. 3). Sediment pesticide concentrations before amendment indicated low levels of background contamination from *S*-metolachlor [1–6 ng g^{-1} (Table 2)] but not atrazine or permethrin. Peak postamendment sediment concentrations of atrazine and *S*-metolachlor varied spatially and temporally with no clear pattern (Table 2), but concentrations were diminished after 22 days. In comparison, permethrin was detected in only one sample at 10 m on day 12 during N + P treatment (Table 2).

Phytoplankton Responses

Because phytoplankton growth rates and metabolism can be influenced by seasonal changes in temperature, hourly measured temperatures in the wetland mesocosm were averaged weekly during both exposure treatment periods and are listed in Supplemental Table 1. Average weekly temperatures in the wetland mesocosm showed nearly identical temperatures in NO or CO during the first treatment exposure period and similar results for temperature in PO and N + P during the second treatment exposure period. In addition, there were minimal differences in average weekly temperatures (typically <4 °C) between the first exposure treatment period (performed July 29 to August 26) and the second exposure treatment period (performed September 2–30). These minimal temperature differences indicated minimal seasonal changes in temperature between the two briefly temporally separated exposure treatment periods with resulting minimal effects on phytoplankton growth rates and metabolism. Thus, comparisons between NO, CO, PO, and N + P were not seasonally biased. Phytoplankton chlorophyll-*a* concentrations were clearly influenced by nutrients during NO treatment as evidenced by large algal blooms occurring 7 days after amendment (Fig. 1). Peak chlorophyll-*a* concentrations during NO occurred earlier than CO treatment (7 days vs. 14–21 days) and were 2.5- to 3.5-fold greater than day 0 pretreatment peaks at each site (Fig. 1). In contrast, chlorophyll-*a* concentrations during PO and N + P did not exhibit such algal blooms but rather showed complex interactions where chlorophyll-*a* concentrations were either unaffected or decreased during these treatments compared with day 0 before treatment (Fig. 2). Forward stepwise linear regression analysis showed complex interactions among components of the varying agrichemical mixtures amended to the wetland that affected phytoplankton chlorophyll-*a* responses (Table 1). Within CO treatment, TN was the most influential variable at all sites, whereas during NO treatment, SRP and DIN strongly influenced chlorophyll-*a* at 10 and 20 m. Negative coefficients for SRP and DIN during NO indicated the time lag between nutrient uptake and population growth in phytoplankton. During PO treatment, chlorophyll-*a* was unaffected by herbicides at 10 m (Table 1), significantly inhibited by herbicides at 20 m, and only moderately affected by pesticides at 40 m (Table 1). In comparison, during N + P, chlorophyll-*a* was significantly inhibited by *S*-metolachlor only at 10 m, moderately affected by atrazine only at 20 m, and minimally affected by *S*-metolachlor only at 40 m (Table 1), thus indicating a possible greater sensitivity to herbicides in the presence of increased nutrient levels.

Crustacean Responses

Water quality measured within the aqueous acute bioassay was within acceptable parameters according to the USEPA (2000) protocol for *H. azteca* reference bioassay tests. Mean (SD) water-quality parameters for the bioassays with field site water were as follows: temperature (°C) 23.4 (0.4); dissolved oxygen (mg L^{-1}) 6.3 (1.4); pH, 8.1 (0.7); alkalinity (mg L^{-1} as $CaCO_3$) 61.3 (9.3); hardness (mg L^{-1} as $CaCO_3$) 138.2 (17.3); and conductivity (μS cm^{-1}) 574.8 (97.1). Preamendment crustacean

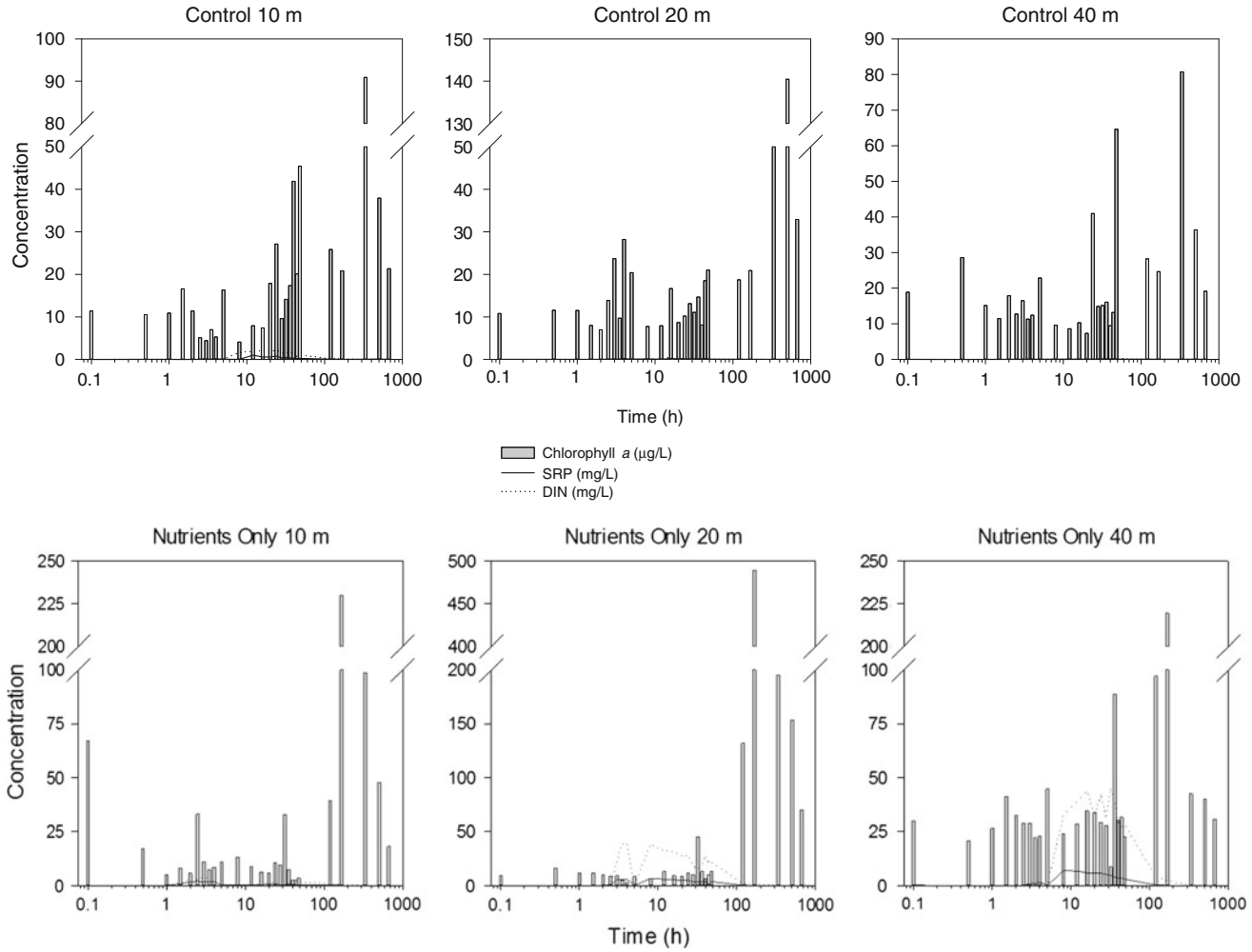

Fig. 1 Chlorophyll-*a*, SRP, and DIN concentrations during CO and NO treatments at 10, 20, and 40 m in a constructed wetland mesocosm

survival in all samples (−1 day) and CO (0 % effluent) was ≥90 % for all 48-h bioassays (Fig. 3; Table 3). No toxicity was observed at any time in wetland effluent from 40 m (Table 3; Fig. 3). Peak wetland effluent toxicity was observed for both PO and N + P treatments within the first 4 h after amendment at 10 and 20 m where *H. azteca* LC_{10} and LC_{50} values ranged from 0.02 to 2.03 % and 0.21 to 59.47 %, respectively (Table 3). Wetland effluent toxicity quickly decreased within 2 days after amendment for both NO and N + P with LC_{50} values >33 % (Table 3), and no toxicity was observed 5 days after amendment at any site (Table 3; Fig. 3). Patterns of crustacean survival in PO treatments was associated with *S*-metolachlor (10 m) and permethrin (10 and 20 m), whereas survival in N + P treatments was associated with nitrate and nitrite (10 m), *S*-metolachlor (10 m), and permethrin (10 and 20 m) (Table 3). Likely sources of toxicity were ascertained using a TU model approach in conjunction with nonlinear logistic or sigmoidal regressions as weight-of-evidence to assess mixture toxicity in both PO and N + P treatments. Regression models for PO treatment showed that permethrin TUs explained >99 % of *H. azteca* survival variation at both sites (Table 3) concomitant with permethrin TU values >1 and *S*-metolachlor TU values <0.05 indicating that permethrin is the primary source of toxicity. Similarly for N + P treatment, regression models showed that permethrin TUs explained >92 % of *H. azteca* survival variation at both sites (Table 3) concomitant with permethrin TU values >1 and N TU and *S*-metolachlor TU values <0.05 again implicating permethrin as the primary source of toxicity.

Sediment bioassay measured water quality was also within acceptable parameters (USEPA 2000). Mean (SD) water quality for bioassays with field site sediment were as follows: temperature (°C) 23.4 (0.3); dissolved oxygen (mg L^{-1}) 7.4 (0.9); pH 8.0 (0.5); alkalinity (mg L^{-1} as $CaCO_3$) 31.4 (12.6); hardness (mg L^{-1} as $CaCO_3$) 68.1 (26.2); conductivity (µS cm^{-1}) 357.7 (45.1); nitrite (mg L^{-1}) 0.08 (0.13); nitrate (mg L^{-1}) 0.60 (0.70); and ammonium (mg L^{-1}) 0.03 (0.03). Crustacean survival was

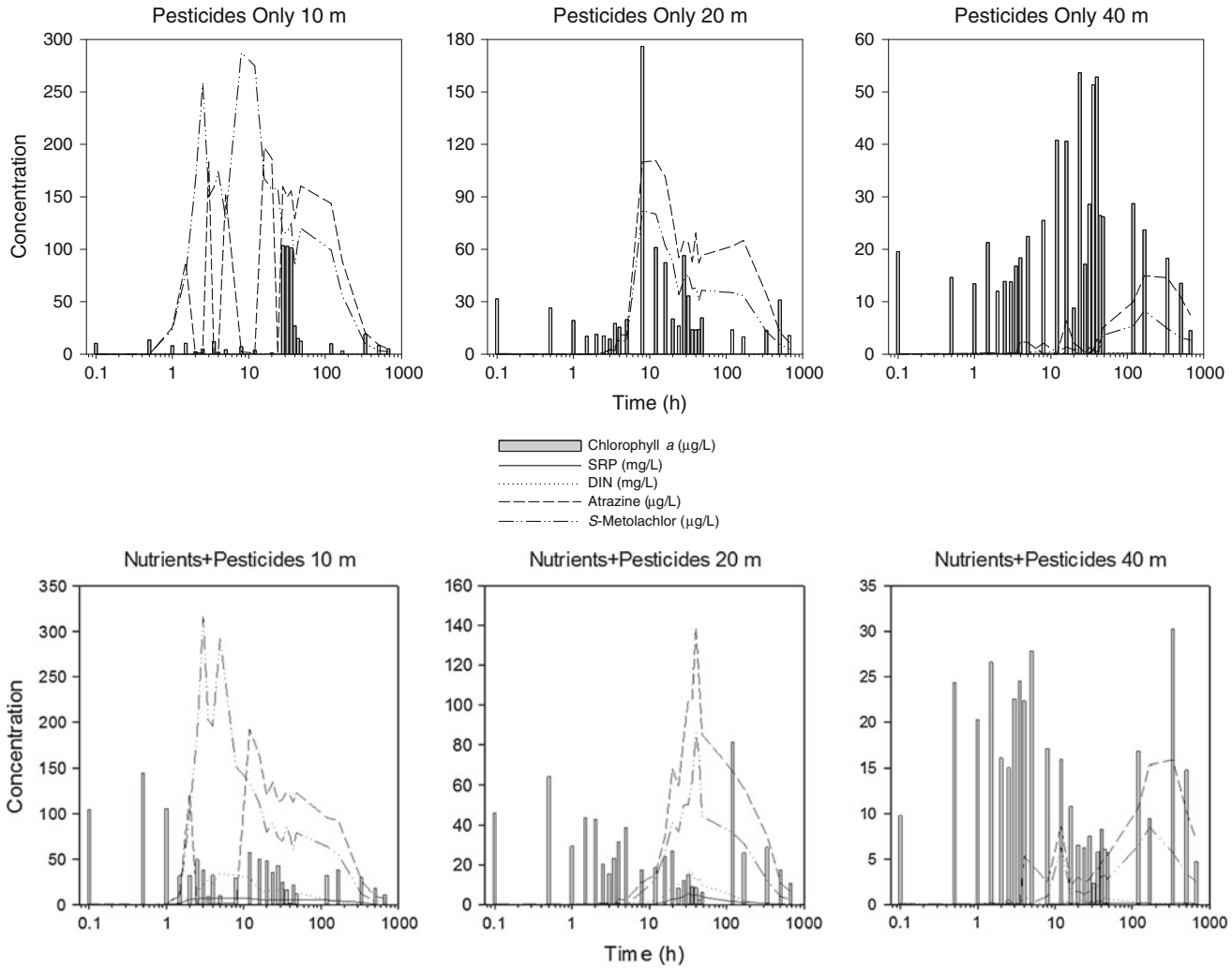

Fig. 2 Chlorophyll-*a*, SRP, and DIN, and atrazine and *S*-metolachlor herbicide concentrations during PO and N + P treatments at 10, 20, and 40 m in a constructed wetland mesocosm

unaffected by sediment exposures at any site during any sampling period with all sites having ≥85 % *H. azteca* survival (Table 2). Significant growth inhibition was observed during N + P treatment with 89 to 98 % decrease in growth at all sites 1 day after amendment. In contrast, during PO treatment, *H. azteca* growth significantly increased from 97 to 157 % at 20 and 40 m 22 days after amendment (Table 2).

Discussion

Currently there is an increasing number of studies on the capabilities of constructed wetlands in trapping contaminants in agricultural run-off and concomitant mitigation of downstream ecological impacts (Schulz and Peall 2001; Schulz et al. 2003; Bouldin et al. 2007; Lizotte et al. 2011). However, gaps in our knowledge exist as to how various agrichemical contaminant mixtures may influence the ability of constructed wetlands to efficiently mitigate ecological impairment, including eutrophication, direct toxicity, and/or indirect toxicity. This study showed that a vegetated constructed wetland under NO conditions will dissipate N and P from the water column in conjunction with an increase in phytoplankton biomass production after a 1-week time lag. Although nutrient soluble fractions would be substantially decreased within the water column, there remained the potential for export of significant algal biomass downstream should the wetland hydraulic retention time coincide with peak algal productivity (Díaz et al. 2012). Although algal blooms after influxes of soluble N and P are well understood (Mitsch and Gosselink 2007), less well known is how constructed wetland hydrology might be managed to minimize the export of large algal biomasses downstream after agricultural inflows. Results of the current study suggest that increasing wetland hydrology by several weeks after soluble N and P influx

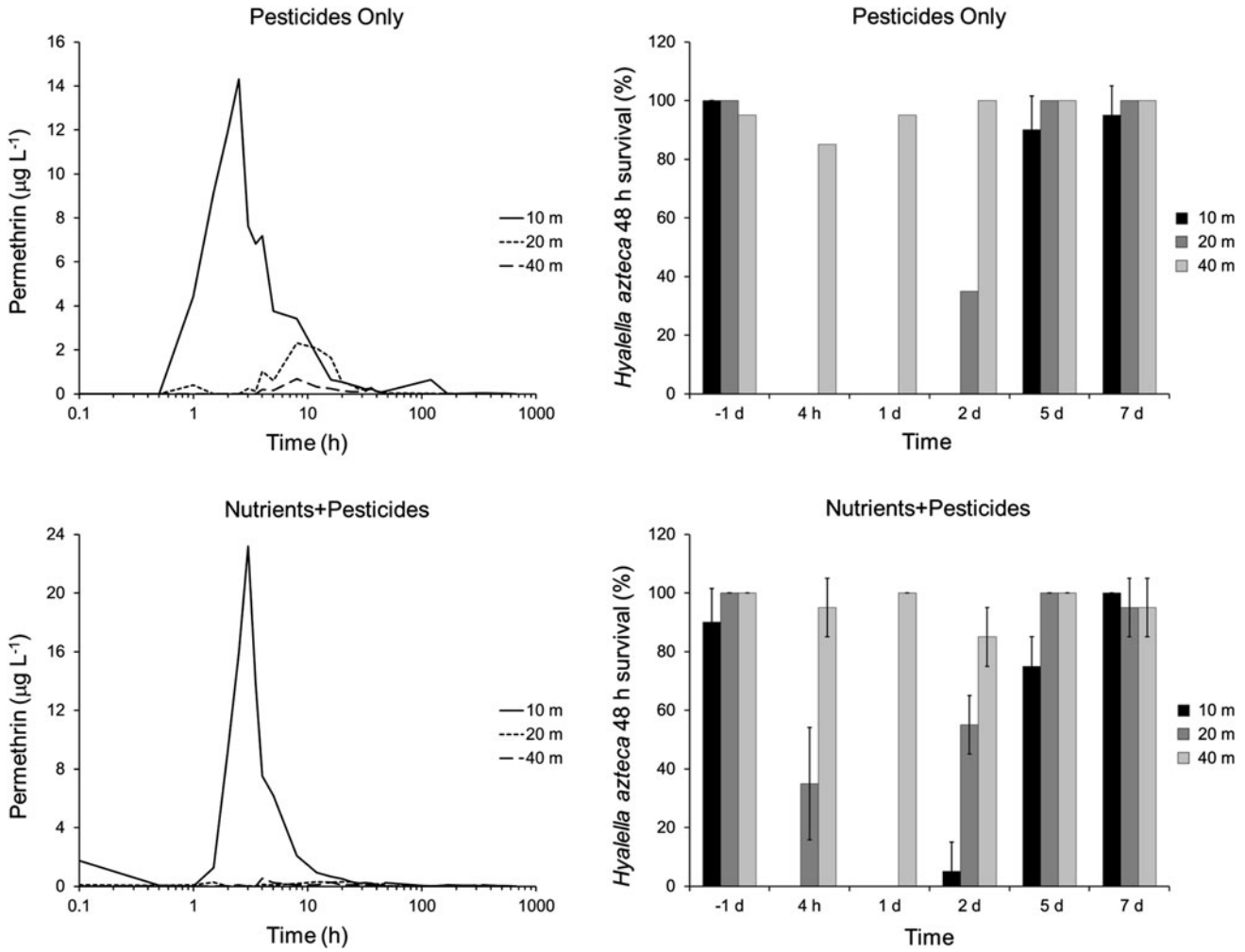

Fig. 3 Permethrin concentrations and *H. azteca* 48-h % aqueous survival ($n = 4$) and SD during PO and N + P treatments at 10, 20, and 40 m in a constructed wetland mesocosm

will greatly lessen the potential for significant export of algal biomass. When phytoplankton was exposed to PO and N + P treatments, these organisms showed more complex responses. During PO treatment, chlorophyll-*a* concentrations at 20 m were rapidly inhibited by atrazine and *S*-metolachlor (within 24 h) with similar responses from chlorophyll-*a* during N + P treatment at 10 and 20 m when herbicide concentrations approached or exceeded reported acute 24-h EC_{50} values for green algae of 125 and 116 µg L^{-1} for atrazine and *S*-metolachlor, respectively (Valloton et al. 2008; Liu and Xiong 2009). Where phytoplankton were exposed to N + P treatment, chlorophyll-*a* concentrations appeared to recover at 20 m after 5 days after amendment (Fig. 2) in conjunction with decreases in herbicide mixture concentrations. Valloton et al. (2008) observed rapid recovery of *Scendesmus vacuolatus* within hours after pulse exposure to atrazine. Such complex responses of phytoplankton chlorophyll-*a* after exposure to different nutrient and herbicide treatments were reported by Waiser and Robarts (1997) where chlorophyll-*a* was suppressed in the presence of herbicides even with nutrients but increased in the presence of nutrients (N + P) alone. Although the total experimental exposure period occurred over a 9-week period, including the first week after the autumnal equinox, seasonal effects were not significant for two reasons. First, changes in light levels during this period had minimal influence on the wetland system dynamics because the light levels were more than sufficient for phytoplankton growth and metabolism (Bellinger and Sigee 2010; Kirk 2011). Second, differences in temperature were minimal with average weekly temperatures ranging from 23.5 to 27.3 °C during the first exposure period and 22.4 to 24.0 °C during the second exposure period. The small (<5 °C) differences in average weekly temperatures between the two exposure periods again indicate that phytoplankton growth and metabolism were not significantly different (Reynolds 2006; Bellinger and Sigee 2010).

Table 2 Sediment *H. azteca* 10-day mean survival (%) ± SD and growth (μg dw) ± SD

Treatment	Distance (m)	Time (days)	End point		Nutrient		Pesticide		
			Survival	Growth	TOC	TN	Atrazine	*S*-metolachlor	Permethrin
Control		−6	92.5 ± 9.6	47.6 ± 28.1	0.62	0.02	BD	BD	BD
		1	92.5 ± 5.0	73.1 ± 27.0	0.47	0.02	BD	1	BD
		5	100 ± 0	62.3 ± 21.1	0.48	0.03	BD	3	BD
		12	100 ± 0	84.8 ± 36.6	0.34	0.01	BD	4	BD
		22	96.9 ± 6.3	58.5 ± 8.1	0.41	0.02	BD	1	BD
PO	10	−6	86.7 ± 11.3	62.2 ± 17.5	1.02	0.06	BD	6	BD
		1	100 ± 0	48.0 ± 17.6	0.31	0.02	21	7	BD
		5	100 ± 0	65.6 ± 29.0	0.58	0.04	3	16	BD
		12	95.0 ± 5.8	108.9 ± 49.4	0.85	0.05	BD	6	BD
		22	97.5 ± 5.0	79.7 ± 14.4	0.47	0.03	BD	3	BD
PO	20	−6	95.0 ± 10.0	63.8 ± 44.3	1.81	0.14	BD	2	BD
		1	85.0 ± 12.9	39.7 ± 13.0	1.23	0.10	4	8	BD
		5	100 ± 0	51.7 ± 33.4	0.98	0.06	30	23	BD
		12	100 ± 0	122.0 ± 34.6	0.60	0.04	3	10	BD
		22	95.2 ± 5.5	163.8 ± 106.5*	0.38	0.03	BD	2	BD
PO	40	−6	97.5 ± 5.0	81.3 ± 16.3	4.26	0.38	BD	2	BD
		1	87.5 ± 9.6	47.2 ± 27.8	5.81	0.54	10	18	BD
		5	95.0 ± 10.0	118.4 ± 33.6	3.92	0.34	36	3	BD
		12	97.5 ± 5.0	86.5 ± 29.5	5.25	0.47	12	20	BD
		22	100 ± 0	160.3 ± 56.7*	5.29	0.47	4	6	BD
N + P	10	−6	97.5 ± 5.0	75.8 ± 19.0	0.38	0.02	BD	6	BD
		1	92.5 ± 9.6	1.7 ± 2.2*	1.56	0.09	BD	37	BD
		5	97.5 ± 5.0	53.5 ± 17.5	0.35	0.02	BD	6	BD
		12	97.5 ± 5.0	104.5 ± 43.5	0.66	0.04	38	16	1
		22	97.5 ± 5.0	57.5 ± 20.0	0.48	0.03	BD	1	BD
N + P	20	−6	95.0 ± 5.8	65.8 ± 20.5	0.45	0.02	BD	BD	BD
		1	95.0 ± 5.8	7.0 ± 8.2*	0.60	0.03	BD	8	BD
		5	100 ± 0	76.7 ± 25.1	0.39	0.02	2	10	BD
		12	97.5 ± 5.0	121.7 ± 37.9	0.42	0.03	BD	2	BD
		22	97.2 ± 5.6	49.1 ± 25.9	0.42	0.3	BD	4	BD
N + P	40	−6	97.5 ± 5.0	78.5 ± 9.9	0.42	0.02	BD	1	BD
		1	90.0 ± 8.2	7.4 ± 5.8*	0.70	0.04	9	9	BD
		5	95.0 ± 10.0	73.5 ± 26.1	0.34	0.02	BD	6	BD
		12	100 ± 0	107.0 ± 38.7	0.37	0.02	BD	2	BD
		22	97.5 ± 5.0	116.2 ± 72.9	0.31	0.02	BD	BD	BD

BD lower than detection limit of 1 ng g^{-1}

* Statistically significantly different from CO, $p < 0.05$, sediment nutrients (%), and sediment pesticides (ng g^{-1}) during varying mixtures of PO or N + P treatments

Crustacean responses to aqueous phase PO and N + P treatments presented clear patterns of toxicity occurring in conjunction with pyrethroid concentrations. Presently several studies have assessed the effects of contaminated agricultural run-off on nontarget aquatic invertebrates in constructed wetlands (Schulz and Peall 2001; Schulz et al. 2003; Bouldin et al. 2007), but these studies focused on responses to insecticide contamination, whereas few studies have attempted to ascertain responses to complex nutrient and insecticide exposures (Lizotte et al. 2011). The present study showed that the observed toxicity to *H. azteca* resulted from exposure to the pyrethroid permethrin with essentially no toxicity from DIN or herbicides as reflected by low TUs (<0.10), which is in agreement with the results of Lizotte et al. (2011) using similar laboratory methods to assess constructed wetland effluent toxicity. In

Table 3 *Hyalella azteca* 48-h aqueous LC_{10} (%) (95 % CIs) and LC_{50} values (%) (95 % CIs) and TUs based on *H. azteca* 96-h LC_{50} values for each nutrient and pesticide listed during varying mixtures of PO or N + P treatments

Treatment	Distance (m)	Time	End point			Toxic units (TUs)					
			LC_{10}	LC_{50}		NH_4^+	NO_2^-	NO_3^-	Atrazine	*S*-metolachlor	Permethrin
PO	10	−1 day	>100	>100		0.00	<0.01	<0.01	<0.01	<0.01	0.00
		4 h	0.02 (< 0.01–0.11)	0.22 (0.01–0.51)		0.00	<0.01	<0.01	<0.01	0.03	194.15
		1 day	0.29 (0.04–0.73)	2.43 (1.07–4.46)		0.00	0.00	<0.01	<0.01	0.03	11.52
		2 day	11.21 (2.68–19.27)	33.27 (19.41–48.07)		0.00	0.00	<0.01	0.11	0.02	10.04
		5 day	>100	>100		0.00	<0.01	<0.01	0.10	0.02	2.70
		7 day	>100	>100		0.00	0.00	<0.01	0.06	<0.01	4.47
					R^2	0.000	0.000	0.000	0.229	0.994*	0.995*
PO	20	−1 day	>100	>100		0.00	0.00	<0.01	<0.01	<0.01	0.00
		4 h	1.27 (0.21–2.49)	4.30 (2.04–6.92)		0.00	0.00	<0.01	<0.01	<0.01	27.90
		1 day	1.52 (0.76–2.36)	6.75 (4.97–8.74)		0.00	0.00	0.00	0.04	<0.01	10.69
		2 day	37.96 (19.83–42.56)	82.78 (52.43–130.68)		<0.01	0.00	<0.01	0.04	<0.01	9.68
		5 day	>100	>100		0.00	<0.01	<0.01	0.04	<0.01	4.06
		7 day	>100	>100		0.00	0.00	0.00	0.04	<0.01	4.45
					R^2	0.270	0.183	0.168	0.183	0.183	0.999*
PO	40	−1 day	>100	>100		0.00	0.00	<0.01	<0.01	0.00	0.00
		4 h	>100	>100		0.00	<0.01	<0.01	<0.01	0.00	5.41
		1 day	>100	>100		0.00	<0.01	<0.01	<0.01	<0.01	2.91
		2 day	>100	>100		0.00	<0.01	<0.01	<0.01	<0.01	5.32
		5 day	>100	>100		0.00	<0.01	<0.01	<0.01	<0.01	5.98
		7 day	>100	>100		0.00	<0.01	<0.01	0.01	<0.01	4.94
					R^2	0.000	0.419	0.893	0.667	0.656	0.000
N + P	10 m	−1 day	>100	>100		0.00	0.00	<0.01	<0.01	<0.01	0.11
		4 h	0.04 (0.04–0.05)	0.21 (0.19–0.25)		0.06	0.01	0.03	<0.01	0.03	203.15
		1 day	0.48 (0.18–0.84)	2.15 (1.36–3.33)		0.08	0.01	0.03	0.09	0.02	8.59
		2 day	6.25 (<0.01–41.05)	57.81 (42.24–67.14)		0.08	0.01	0.02	0.08	0.01	12.61
		5 day	>100	>100		0.07	<0.01	<0.01	0.06	0.01	5.09
		7 day	>100	>100		0.07	<0.01	<0.01	0.06	<0.01	7.35
					R^2	0.210	0.990*	0.992*	0.000	0.991*	0.936*
N + P	20 m	−1 day	>100	>100		0.00	0.01	<0.01	<0.01	<0.01	0.07
		4 h	2.03 (0.33–5.04)	59.47 (35.48–114.49)		<0.01	<0.01	<0.01	<0.01	<0.01	14.92
		1 day	3.54 (2.11–5.37)	14.55 (9.87–21.87)		0.04	<0.01	0.02	0.04	<0.01	8.07
		2 day	1.96 (0.53–4.17)	>100		0.07	0.01	0.01	0.05	<0.01	4.96
		5 day	>100	>100		0.05	<0.01	<0.01	0.04	<0.01	3.89
		7 day	>100	>100		0.04	<0.01	<0.01	0.04	<0.01	1.94
					R^2	0.000	0.113	0.647	0.180	0.471	0.926*
N + P	40 m	−1 day	>100	>100		0.00	0.00	0.00	<0.01	<0.01	0.23
		4 h	>100	>100		0.00	0.00	<0.01	<0.01	<0.01	3.49
		1 day	>100	>100		<0.01	<0.01	<0.01	<0.01	<0.01	2.87
		2 day	>100	>100		<0.01	<0.01	<0.01	<0.01	<0.01	4.05
		5 day	>100	>100		<0.01	<0.01	<0.01	<0.01	<0.01	4.52
		7 day	>100	>100		<0.01	<0.01	<0.01	0.01	<0.01	4.48
					R^2	0.000	0.275	0.891	0.364	0.364	0.364

Coefficients of determination (R^2) generated using nonlinear sigmoidal or logistic regressions ($n = 6$) for assessing relationships between nutrient or pesticide toxic units (independent variable) and wetland *Hyalella azteca* 48-h mortality (dependent variable)

* Statistically significant, $p \leq 0.05$

contrast, *H. azteca* responses to sediment phase during PO and N + P treatments elucidated no clear patterns of toxicity in conjunction with nutrient and/or pesticide sediment concentrations. Although fewer studies have assessed sediment toxicity from contaminated agricultural run-off on nontarget aquatic invertebrates in constructed wetlands

(Bouldin et al. 2007), the results of the present study concur with those of Bouldin et al. (2007) showing limited, if any, consistent responses from benthic organisms after amendment.

Constructed wetlands can rapidly and efficiently trap and process agricultural run-off& comprised of a variety of pollutant mixtures. Such wetlands can also mitigate ecological impacts to receiving aquatic systems from nutrients (by way of eutrophication) and pesticides (by way of direct toxicity). Mitigation of ecological effects occurs rapidly within days for permethrin, days to weeks for herbicides and weeks for nutrients. However, the wetland ecosystem itself undergoes changes in community structure during exposure to the run-off and event due to eutrophication during NO or direct toxicity during PO and N + P treatments.

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