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Do Varying Aquatic Plant Species Affect Phytoplankton and Crustacean Responses to a Nitrogen-Permethrin Mixture?

Richard E. Lizotte Jr[1] · Matthew T. Moore[1]

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Abstract Hydraulically connected wetland microcosms vegetated with either *Typha latifolia* or *Myriophyllum aquaticum* were amended with an NH_4NO_3 and permethrin mixture to assess the effectiveness of both plant species in mitigating effects of the pollutant mixture on phytoplankton (as chlorophyll *a*) and *Hyalella azteca*. Phytoplankton grew in response to increased NH_4NO_3 in the presence of all plant species, but was unaffected by exposure to permethrin. *H. azteca* responses occurred rapidly (0.17 days), was mitigated within 1–2 days, and aqueous toxicity was unaffected by plant species type. A toxic unit model approach ascertained primary toxicity was permethrin with minimal additional toxicity from NH_4NO_3. Varying aquatic plant species had only modest influences on phytoplankton responses and no observable influence on animal responses during nitrogen-permethrin mixture exposures. As a result, both *T. latifolia* and *M. aquaticum* can be used as part of an effective agricultural best-management practice system for mitigating pollutant impacts of agricultural run-off.

Keywords *Myriophyllum Typha* · NH_4NO_3 · Permethrin · Chlorophyll *a* · *Hyalella azteca*

Agricultural non-point source runoff is a significant contributor to surface water impairment globally (Parris 2011) and is often comprised of a mixture of pollutants, including nutrients and pesticides. Insecticides such as the pyrethroid permethrin have been observed to be transported in runoff from agricultural fields and enter aquatic systems at concentrations ranging from 0.6 to greater than 2 µg/L (Delgado-Moreno et al. 2011; Werner et al. 2010), potentially impacting aquatic biota. Nitrogen concentrations in runoff resulting from fertilizer applications have been observed to be in excess of 20 mg/L with some concentrations >30 mg/L (Gentry et al. 2000; Randall et al. 2003). Significant research has been conducted in an attempt to ascertain agricultural best management practices (BMPs) that minimize pollutant transport and associated ecological impacts (Hunt et al. 2008; Lizotte et al. 2011; Schulz and Peall 2001). Best management practices utilizing aquatic plants have been shown to effectively mitigate pollution in agricultural runoff (Moore et al. 2009, 2013; Moore and Kröger 2011; Tyler et al. 2012). A study by Moore et al. (2013) showed that different aquatic plants can have significantly different effectiveness in mitigating pesticides such as permethrin. Also Tyler et al. (2012) demonstrated that different aquatic plants can also remove dissolved nitrogen with different efficiencies. Two plant species of vegetation types that have been suggested for use or introduction to constructed and/or restored wetlands are the common cattail (*Typha latifolia* L.) and milfoil [*Myriophyllum aquaticum* (Vellozo) Verdcourt] (Mitsch and Gosselink 2007). *T. latifolia* is categorized as a tall perennial erect emergent aquatic macrophyte with large, creeping rhizomes and often densely colonial growing up to 3 m tall (Rejmánková 1992; Bryson and DeFelice 2009). *M. aquaticum* is categorized as a creeping emergent hydrophyte that roots in the substrate and produces dense tangled vegetated mats. Milfoil has long stems that are predominantly submerged in the water column with only the reproductive or fruiting tip emerging from the water surface (Rejmánková 1992). Because of their life history, both plant species have the potential for use in managing agricultural runoff laden with

✉ Richard E. Lizotte Jr
richard.lizotte@ars.usda.gov

[1] USDA-ARS National Sedimentation Laboratory, Oxford, MS, USA

nutrients and pesticides. However, little research has been done to determine how effective different plant species are in mitigating ecological effects of agriculturally derived pollution. To attempt to fill this gap, the goal of the present study was to assess the potential for two different aquatic plant species, *T. latifolia* and *M. aquaticum* singly or in combination, in mitigating the aqueous ecological effects of an ammonium nitrate-permethrin mixture on phytoplankton chlorophyll *a* and crustacean *Hyalella azteca*.

Materials and Methods

The study was conducted with eight sets of two hydraulically connected 115 L wetland microcosms (16 total) in series to provide four combinations of two aquatic plant types with four replicates of the following combinations: upstream microcosms, *Typha latifolia* only (T) and *Myriophyllum aquaticum* only (M); and upstream into downstream microcosms, *Typha latifolia* into *Myriophyllum aquaticum* (TM); and *Myriophyllum aquaticum* into *Myriophyllum aquaticum* (MM). Each microcosm had sand and silty loam sediment (0.06 m^3) for root growth and an average surface water holding capacity of about 44–54 L at an average water depth of 17–21 cm (Fig. 1). Average measured above-ground biomass for each vegetation type was as follows: T = 250.2 ± 78.4 g; M = 109.7 ± 86.3 g dw; TM = 73.6 ± 22.0 g dw; and MM = 173.4 ± 76.9 g dw. During 4 h dosing, 95–96 L of water amended with a mixture of nitrogen (8 g of 34% NH_4NO_3) and permethrin (0.36 mg active ingredient of Hi Yield 38®) was pumped at a flow rate of approximately 24 L/h using Fluid Metering Inc. (FMI™) piston pumps (Fluid Metering Inc., Syosset, NY, USA) into the eight upstream wetland microcosms to simulate a "first flush" runoff event. Surface water was sampled at the downstream end of each microcosm for dissolved inorganic nitrogen (DIN) and chlorophyll *a* (230 mL), permethrin (500 mL), and laboratory crustacean *Hyalella azteca* (230 mL) bioassays. Water was collected from each wetland microcosm at −7 days (pretreatment), 0.17 days (4 h), 1, 2, 3, 4, and 7 days post-amendment for nitrogen, chlorophyll *a*, and permethrin while sampling for aqueous bioassays occurred on −7 days, 0.17 days, 1 and 2 days.

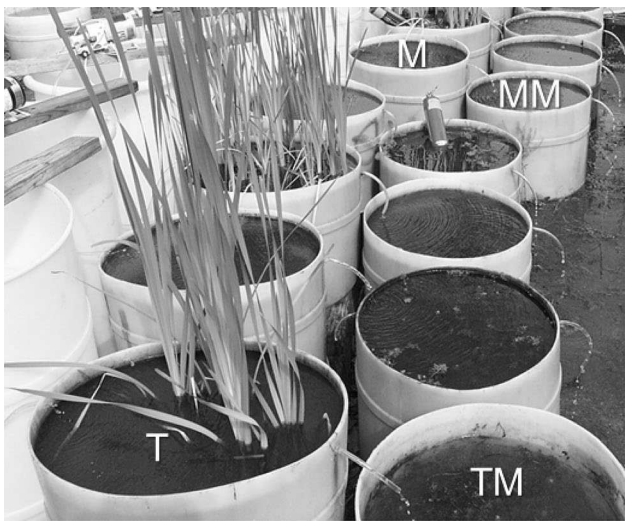

Fig. 1 Experimental wetland microcosms vegetated with: *Typha latifolia* only (T); *Typha latifolia* into *Myriophyllum aquaticum* (TM); *Myriophyllum aquaticum* only (M); and *Myriophyllum aquaticum* into *Myriophyllum aquaticum* (MM) treatments

Analysis of inorganic nitrogen, ammonium and nitrate, were conducted spectrophotometrically on 0.45 µm filtered samples using the phenate method and cadmium reduction method, respectively, according to APHA (2005) with a detection limit of 0.02 mg/L for both inorganic N species. Analysis of permethrin was conducted using a method outlined by Smith et al. (2007) where permethrin was extracted using analytical-grade ethyl acetate. Next, the extract was dried and concentrated via rotary evaporation over anhydrous Na_2SO_4 followed by silica gel column cleanup and further concentration to 1 mL under high purity dry N for gas chromatography (GC) analysis using concurrent Agilent HP model 6890 GCs (Agilent Technologies, Inc., Waldbronn, Germany). Both *cis*- and *trans*-permethrin isomers were analyzed via GC with recoveries and extraction efficiencies, based on fortified samples, ≥90% for both isomers and having detection limits of 0.05 µg/L for both isomers.

Phytoplankton biomass as chlorophyll *a* was analyzed from water samples collected congruently with nutrient and pesticide analysis using the trichromatic pigment extraction method (APHA 2005). Briefly, Micro Filtration Systems (MFS; Micro Filtration Systems, Dublin, CA, USA) 0.45 µm cellulose nitrate filters were used to collect water column phytoplankton from samples. Chlorophyll *a* was extracted from the filter-collected phytoplankton via freezing and acetone extraction followed by spectrophotometric determination using a ThermoSpectronic Genesys 10 ultraviolet (UV) spectrophotometer (Spectronic Instruments, Inc., NY, USA). Crustacean *H. azteca* 48 h static non-renewed aqueous laboratory bioassays measuring mortality were conducted following procedures outlined by USEPA (2000, 2002). Bioassay sampled water was hardness adjusted to about 100 mg $CaCO_3$ L^{-1} using $CaCl_2$ and $NaHCO_3$, and distributed in 88 mL test chambers for whole effluent toxicity bioassays. *H. azteca* were collected from cultures located at the USDA-ARS laboratory, Oxford, Mississippi, via passing through a 600 µm stainless steel mesh sieve but retained by a 425 µm stainless steel mesh sieve (approximately 1–2 weeks old). Five *H. azteca* were added to each of four replicate 88 mL polypropylene

plastic test chambers containing 75 mL effluent and one 2 cm × 2 cm square sterile cotton gauze as substrate. Aqueous exposures consisted of whole effluent with five serial dilutions at 0.25 dilution factor. Control and dilution water, free from priority pollutants, were from a naturally spring-fed pond located at the University of Mississippi Field Station (UMFS), Bay Springs, Mississippi, and also hardness adjusted. Bioassays were conducted in an environmental incubator (Powers Scientific, Inc., Pipersville, PA, USA) at 23 ± 1°C with a photoperiod of 16:8 light:dark. Physicochemical characteristics of temperature, dissolved oxygen, pH, alkalinity, hardness, conductivity, nitrite, nitrate, and ammonium were measured using standard methods (APHA 2005).

Data were analyzed using several statistical methods using either Toxcalc v5.0.32 (ToxCalc 2008) or SigmaPlot v12.0 statistical software (SYSTAT 2011) packages. Two-way repeated-measure analysis of variance (ANOVA) was conducted to assess effects of both time and plant type on phytoplankton biomass and *H. azteca* mortality using SigmaPlot v12.0 (SYSTAT 2011). Non-linear exponential regressions were conducted for phytoplankton biomass over time to determine chlorophyll *a* daily rate of increase (growth rate). Pearson Product Moment correlation analysis was conducted to assess relationships of phytoplankton biomass versus water depth, temperature, pH, nutrients (including total phosphorus), and permethrin concentration using SigmaPlot v12.0 (SYSTAT 2011). *H. azteca* 48-h mortality analysis was conducted using ToxCalc v5.0.32 (ToxCalc 2008) to estimate LC10 and LC50 effluent dilution fraction effects. Acute (48–96 h) toxicity units (TUs), the measured concentration divided by the median lethal concentration described by Pape-Lindstrom and Lydy (1997) were calculated for NH_4-N (39.8 mg/L; Ankley et al. 1995), NO_3-N (124.2 mg/L; Pandey et al. 2011), and permethrin (0.037 µg/L; Wheelock et al. 2005) components of the mixture. Non-linear sigmoidal three parameter regressions were performed on TUs versus *H. azteca* mortality to elucidate likeliest sources of observed toxicity using SigmaPlot v12.0 statistical software (SYSTAT 2011).

Results and Discussion

Peak concentrations of amended DIN (NH_4-N, NO_3-N) and permethrin isomers occurred within 0.17 days of amendment and rapidly dissipated from the water column within the 7-day study period (Table 1). By 7 days, NH_4-N decreased by 93% in T, 61% in TM, 58% in M, and 76% in MM plant types. Comparably, by 7 days, NO_3-N decreased by 90% in T, 74% in TM, 67% in M, and 83% in MM plant types. Permethrin isomers dissipated from the water column much more rapidly than DIN. *Cis*-permethrin decreased by >99% in T, >99% in TM, 98% in M,

Table 1 Mean measured aqueous concentrations of nitrogen (mg/L) and permethrin (µg/L) in wetland microcosms with: *Typha latifolia* only (T); *Typha latifolia* into *Myriophyllum aquaticum* (TM); *Myriophyllum aquaticum* only (M); and *Myriophyllum aquaticum* into *Myriophyllum aquaticum* (MM)

Day	NH_4-N				NO_3-N			
	T	TM	M	MM	T	TM	M	MM
−7	0.03	0.02	0.02	0.05	1.50	1.34	0.97	0.93
0.17	32.93	21.35	31.78	23.63	37.44	29.00	36.66	29.61
1	25.15	18.23	29.13	15.23	33.63	27.40	36.83	23.55
2	18.95	16.88	26.08	13.95	22.77	19.90	26.70	17.48
3	13.65	15.55	25.05	12.08	17.11	17.77	23.43	12.28
4	9.49	14.20	22.40	10.59	14.61	14.21	26.54	14.21
7	2.38	8.32	13.32	5.62	3.80	7.65	12.24	5.15
Day	*Cis*-permethrin				*Trans*-permethrin			
	T	TM	M	MM	T	TM	M	MM
−7	B[a]	B	B	B	B	B	B	B
0.17	1.27	0.60	1.03	0.75	1.03	0.44	0.77	0.48
1	B	0.06	B	B	B	B	B	B
2	B	B	B	B	B	B	B	B
3	0.14	B	B	B	B	B	B	B
4	B	B	B	B	B	B	B	B
7	0.11	0.27	B	0.15	B	B	B	B

[a] *B* below detection limit of 0.05 µg permethrin/L aqueous

and >99% in MM plant types by 2 days. Similarly, *Trans*-permethrin decreased by >99% in T, 96% in TM, 98% in M, and >99% in MM plant types by 2 days. However, both permethrin isomers had fluctuating concentrations from days 3–7 likely due to desorption from surfaces such as plant stems/leaves and microcosm walls. Sediment desorption was an unlikely source since permethrin was below detection limits for any sample measured during the experiment (Table 1). Several studies have shown that aquatic vegetation is very effective at mitigating either nitrogen or permethrin (Moore et al. 2009, 2013; Moore and Kröger 2011; Tyler et al. 2012) but fewer studies have examined more complex mixtures of both nutrients and pesticides simultaneously (Lizotte et al. 2011).

Despite the presence of aquatic plants, phytoplankton chlorophyll *a* showed significant increases in all microcosms, regardless of plant species (Fig. 2). Results of the two-way repeated-measures ANOVA with time ($p=0.003$), plant type ($p=0.334$), and time X plant type interaction ($p=0.031$) showed significant temporal variations with interactions between both time and plant type. Average phytoplankton growth rates among plant treatments were $T=0.33\pm0.09$ mg chlorophyll *a*/d; $M=0.27\pm0.17$ mg chlorophyll *a* /d; $TM=0.21\pm0.09$ mg chlorophyll *a*/d; and $MM=0.23\pm0.11$ mg chlorophyll *a*/d. Only by 7 days were significant differences in chlorophyll *a* among plant treatments observed with M = T > TM, MM, where T had the greatest average growth in phytoplankton but not statistically significantly greater than M. Correspondingly, phytoplankton in T had the highest growth rates and coefficient of determination (R^2) (Fig. 2). Observed differences in phytoplankton growth between *T. latifolia*-planted microcosms and two of the three *M. aquaticum*-planted microcosms can be explained by several possible conditions. It has been commonly observed that dense growth of submerged or creeping aquatic plants such as *M. aquaticum* suppress

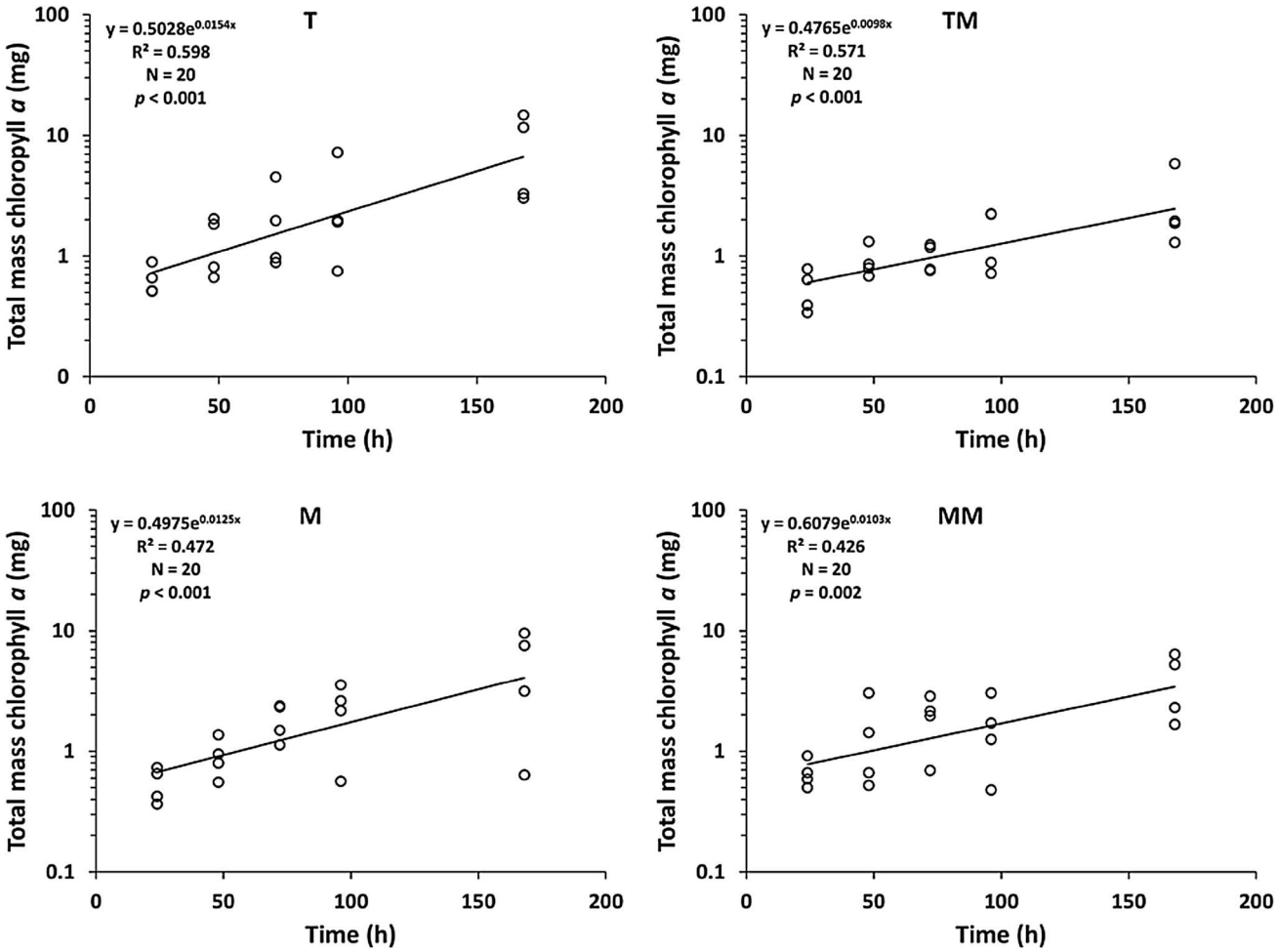

Fig. 2 Exponential growth of phytoplankton (mg chlorophyll *a*) in microcosms vegetated with: *Typha latifolia* only (T); *Typha latifolia* into *Myriophyllum aquaticum* (TM); *Myriophyllum aquaticum* only (M); and *Myriophyllum aquaticum* into *Myriophyllum aquaticum* (MM) treatments

phytoplankton growth (Gopal and Goel 1993). Such growth suppression can occur through direct competition between the aquatic plant and phytoplankton or indirect competition between the aquatic plant epiphytes (periphyton) and phytoplankton for resources such as light and nutrients (Rejmánková 1992; Gopal and Goel 1993). Another method of suppression is through allelopathy (Gopal and Goel 1993), however, the exact nature of phytoplankton growth suppression in the presence of *M. aquaticum* is beyond the scope of the current study.

Correlation analysis revealed significant correlations between chlorophyll *a* and temperature, total phosphorus, and DIN (Table 2). High negative correlation coefficients (Taylor 1990) between chlorophyll *a* and both NH_4-H and NO_3-N were observed for T and M while more modest coefficients (Taylor 1990) occurred for TM and MM (Table 2). These results indicate some uptake and utilization of DIN with ensuing phytoplankton growth during the 7-day study (Reynolds 2006) in competition with co-existing aquatic plants (Gopal and Goel 1993). The lack of significant correlations between chlorophyll *a* mass and either permethrin isomers indicate permethrin concentrations were not sufficient to elicit a phytoplankton growth response (Stratton and Corke 1982).

Aqueous survival responses of the crustacean, *H. azteca*, clearly indicated effects of the NH_4NO_3-permethrin mixture. Pre-dosing (−7 days) and control (0% effluent) crustacean survival was ≥90% for all 48-h bioassays (Table 3). Within 0.17 days, no animals survived in 100% effluent.

H. azteca survival across time with −7 days >0.17 days <1 days <2 days indicated that greatest mortality occurred at 0.17 days and mortality was ameliorated by 1–2 days post-dosing. Possible sources of crustacean toxicity were determined using a sigmoidal regression TU model approach as weight-of-evidence to assess mixture toxicity. Sigmoidal regression models showed *H. azteca* mortality was not explained by NH_4-N and only moderately explained by NO_3-N ($R^2 = 0.393$; Taylor 1990) in addition to having TU values <1.0 (Fig. 3). These results indicate that NH_4NO_3 contributed minimally to the observed crustacean mortality in microcosm effluents. In contrast, the TU permethrin model explained 81.9% of *H. azteca* mortality with ΣTU model providing only 0.2% better explanatory power (82.1%) as well as permethrin TU values >>10 indicating extremely toxic permethrin concentrations and implicating permethrin as the primary toxicant in the mixture. While several previous studies have examined ecological effects of nutrient-pesticide mixtures in vegetated systems (Hunt et al. 2008; Lizotte et al. 2011; Schulz and Peall 2001) there exists a paucity of information regarding how different aquatic plant species might influence pesticide toxicity to invertebrates. Results of the current study clearly indicate that neither *T. latifolia* nor *M. aquaticum*

Table 2 Pearson-Product Moment correlation coefficients (*r*) of $\log_{10}$ phytoplankton chlorophyll *a* (n = 20) versus physical and chemical water quality variables in wetland microcosms with: *Typha latifolia* only (T); *Typha latifolia* into *Myriophyllum aquaticum* (TM); *Myriophyllum aquaticum* only (M); and *Myriophyllum aquaticum* into *Myriophyllum aquaticum* (MM)

Physical, chemical water quality variable	Phytoplankton chlorophyll *a* (mg)			
	T	TM	M	MM
Depth (cm)	−0.097	0.361	−0.101	0.296
Temperature (°C)	−0.639*	−0.599*	−0.510*	−0.542*
pH	−0.226	0.273	−0.392	0.378
Total P (mg/L)	0.401	0.525*	0.553*	0.588*
NH_4-N (mg/L)	−0.769*	−0.640*	−0.665*	−0.553*
NO_3-N (mg/L)	−0.737*	−0.629*	−0.754*	−0.572*
Cis-permethrin (µg/L)	0.138	0.266	−0.337	0.095
Trans-permethrin (µg/L)	−0.234	−0.271	0.037	−0.179

*Statistically significant, $p \leq 0.05$

Table 3 *Hyalella azteca* 48-h aqueous mean (SD) whole effluent survival (%), LC10s (%) (95% confidence intervals), and LC50s (%) (95% confidence intervals) exposed to NH_4NO_3 and permethrin with: *Typha latifolia* only (T); *Typha latifolia* into *Myriophyllum aquaticum* (TM); *Myriophyllum aquaticum* only (M); and *Myriophyllum aquaticum* into *Myriophyllum aquaticum* (MM)

Time (days)	Endpoint	Vegetation type			
		T	TM	M	MM
−7	Survival	95 (10)	95 (10)	100 (0)	90 (12)
	LC10	>100	>100	>100	>100
	LC50	>100	>100	>100	>100
0.17	Survival	0 (0)	0 (0)	0 (0)	0 (0)
	LC10	1.6 (0.8–2.3)	1.9 (1.2–2.8)	2.4 (0.1-4.0)	4.2 (1.6–6.3)
	LC50	3.8 (2.7–5.4)	7.6 (5.5–10.5)	5.3 (2.1–7.4)	9.2 (6.1–13.3)
1	Survival	70 (48)	95 (10)	80 (16)	65 (44)
	LC10	>100	>100	>100	>100
	LC50	>100	>100	>100	>100
2	Survival	100 (0)	100 (0)	100 (0)	100 (0)
	LC10	>100	>100	>100	>100
	LC50	>100	>100	>100	>100

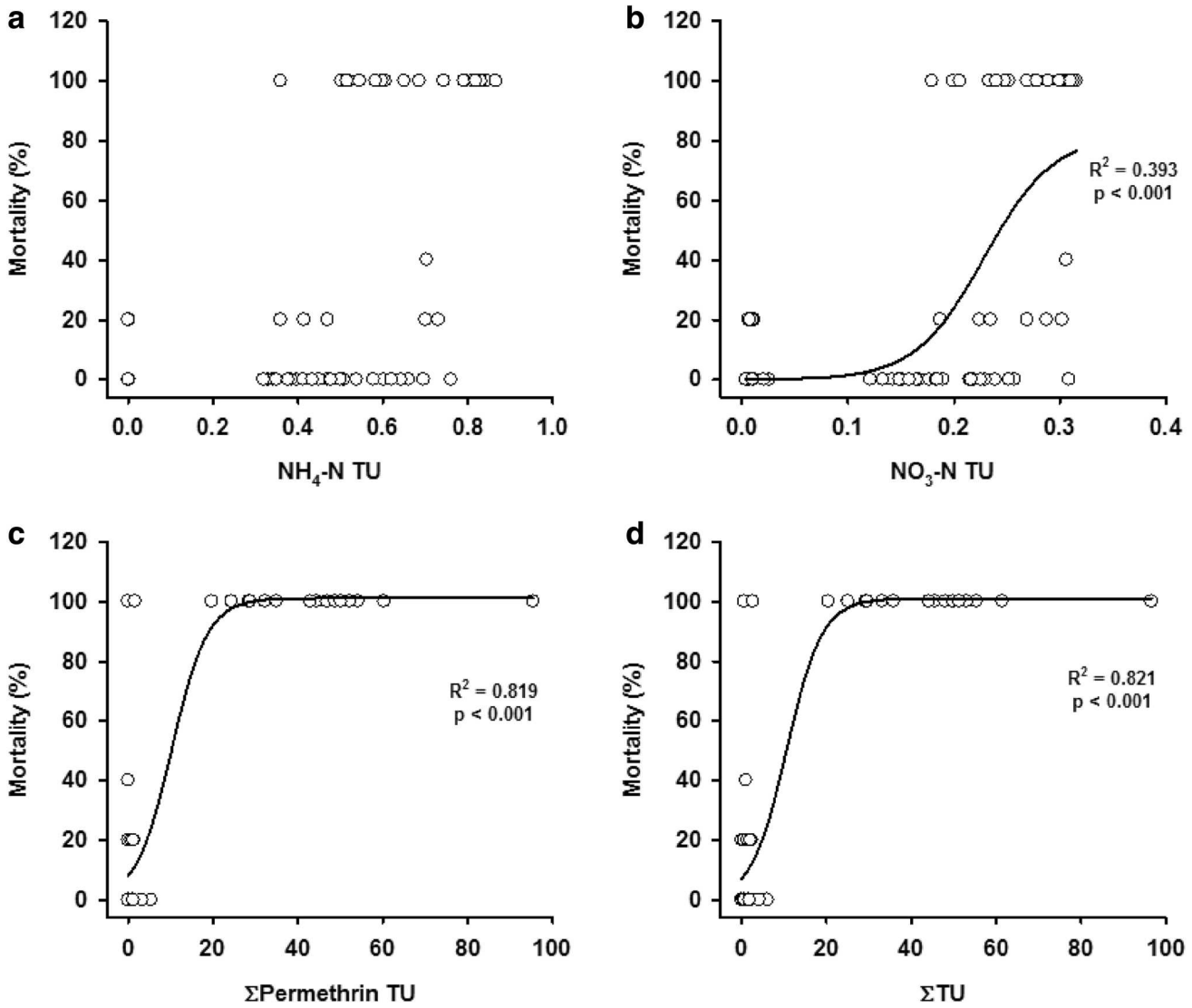

Fig. 3 Non-linear sigmoidal regression relationships (N = 64) between *Hyalella azteca* 48-h mortality and **a** NH_4-N TUs, **b** NO_3-N TUs, **c** ΣPermethrin TUs, and **d** ΣTUs in wetland microcosms vegetated with either *Typha latifolia* or *Myriophyllum aquaticum*

affected acute aqueous permethrin toxicity to *H. azteca* and that permethrin toxicity to aquatic invertebrates was eventually equally mitigated by both plant species.

This study showed that varying aquatic plant species had only modest influences on phytoplankton responses where growth was greatest in *T. latifolia* microcosms by 7 days and was unaffected by exposure to permethrin. In comparison, there was no observable influence of varying plant species on mitigating *H. azteca* aqueous toxicity during nitrogen-permethrin mixture exposures. As a result, both species of aquatic macrophytes, the emergent macrophyte, *T. latifolia* and the submerged macrophyte, *M. aquaticum* can be used as part of an effective agricultural best-management practice for mitigating pollutant impacts of agricultural run-off.

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