

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere



Using aquatic vegetation to remediate nitrate, ammonium, and soluble reactive phosphorus in simulated runoff



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HIGHLIGHTS

- Typha latifolia decreased soluble reactive phosphorus concentrations in runoff.
- Panicum hemitomon decreased nitrate concentrations in year one, but not year two.
- Mixtures of macrophytes may likely increase nutrient retention efficiency.

ARTICLE INFO

Article history: Received 10 May 2016 Received in revised form 15 June 2016 Accepted 20 June 2016 Available online 30 June 2016

Handling Editor: Shane Snyder

Keywords: Phytoremediation Mesocosm Wetland Nitrogen Phosphorus

ABSTRACT

Within the agriculturally-intensive Mississippi River Basin of the United States, significant conservation efforts have focused on management practices that reduce nutrient runoff into receiving aquatic ecosystems. Only a small fraction of those efforts have focused on phytoremediation techniques. Each of six different aquatic macrophytes were planted, in monoculture, in three replicate mesocosms (1.2 m \times 0.15 m \times 0.65 m). Three additional unvegetated mesocosms served as controls for a total number of 21 mesocosms. Over two years, mesocosms were amended once each summer with sodium nitrate, ammonium sulfate, and potassium phosphate dibasic to represent nitrogen and phosphorus in agricultural runoff. System retention was calculated using a simple aqueous mass balance approach. Ammonium retention in both years differed greatly, as Panicum hemitomon and Echinodorus cordifolius retentions were significantly greater than controls in the first year, while only Myriophyllum aquaticum and Typha latifolia were significantly greater than controls in the second year. Greater soluble reactive phosphorus retention was observed in T. latifolia compared to controls in both years. Several other significant differences were observed in either the first or second year, but not both years. In the first year's exposure, P. hemitomon was significantly more efficient than the control, Saururus cernuus, and T. latifolia for overall percent nitrate decrease. Results of this novel study highlight inherent variability within and among species for nutrient specific uptake and the temporal variations of species for nutrient retention. By examining this natural variability, scientists may design phytoremediation systems with greater impact on improving agricultural runoff water quality.

Published by Elsevier Ltd.

1. Introduction

With the US population expected to top 322 million people at the beginning of 2016, and more than seven billion global inhabitants, there will continue to be an intensification of food and fiber production to meet national and international needs (US

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Census Bureau, 2015). Increased agricultural production will require increases in application of nitrogen (N) and phosphorus (P), through either inorganic fertilizer or manure, to sustain and increase yields in agriculture. Howarth et al. (2002) identified inorganic fertilizers as the single largest global source of reactive N. In 2011, approximately 11.6 million Mg of N and 3.92 million Mg of P were applied to US crops (ERS, 2015; Lerch et al., 2015). It has been estimated that approximately 50% of all N compounds applied in agricultural settings do not reach the intended target and are lost to the surrounding environment (Davidson et al., 2012.) As a result, multiple studies suggest not only is agriculture the largest source of N compounds entering the environment, but N is also the single

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most significant environmental pollutant produced by agriculture (Ribaudo, 2011; Godfray, 2014). Giles (2005) went so far as to suggest that, aside from biodiversity loss and climate change, N pollution is the third largest threat to the planet's existence.

Excessive nutrients can leave the production landscape and enter surrounding aquatic ecosystems, potentially resulting in eutrophication. Eutrophication can lead to zones of hypoxia, causing significant environmental and economic damage. Dodds et al. (2009) estimated the annual total ecological and economic costs of freshwater eutrophication in the US alone at \$2.2 billion. Although the hypoxia concern within the Gulf of Mexico garners much of the national and international attention, there are also approximately 300 hypoxic zones along the entire US coastline (Davidson et al., 2012). To address these serious issues, agricultural policies and support systems must include analyses of innovative best management practices (BMPs) to reduce nutrient loss from the production landscape.

Various BMPs have been suggested to remediate nutrients associated with storm or irrigation runoff. Many of these practices utilize vegetation, whether near field edges as constructed wetlands, riparian buffers, or drainage ditches, or in fields as cover crops or reduced tillage. In the intensively farmed lower Mississippi River Valley states of Arkansas, Mississippi, and Louisiana, ditches drain 1,244,543 ha; 672,546 ha; and 969,331 ha of agricultural land, respectively (USDA, 2012). Several studies have highlighted opportunities for drainage ditch management to decrease nutrient concentrations from agricultural runoff through combinations of biogeochemical and physical processes (Kröger et al., 2007, 2008; Strock et al., 2007; Moore et al., 2010). Increased efforts are focused on identifying specific plant species that will provide the greatest remediation potential in a multitude of different environmental circumstances. Successful nutrient phytoremediation is dependent on many interacting factors between the plants, water quality (e.g. temperature, redox, pH, etc.), flow conditions, and nutrient load. The objective of the current study was to examine the individual ability of six emergent macrophytes [Typha latifolia L. (broad-leaved cattail), Panicum hemitomon Schult. (maidencane), Thalia dealbata Fraser ex Roscoe (powdery alligator-flag), Echinodorus cordifolius (L.) Griseb.(creeping burhead), Myriophyllum aquaticum (Vell.) Verdc. (parrot feather), and Saururus cernuus L. (lizard's tail)] to mitigate N and P concentrations in simulated storm runoff water over two summer seasons.

2. Materials and methods

Mesocosms were constructed using 21 Durapride™ high density polyethylene oval containers (1.2 m \times 0.15 m \times 0.65 m), with a 22 cm base of sand overlain with 16 cm of Lexington silt loam. Mesocosms were planted with monocultures of one of the following six rooted, emergent macrophytes: T. latifolia L., P. hemitomon, T. dealbata, E. cordifolius, M. aquaticum, and S. cernuus. Sand and silt loam used as mesocosm substrate were collected from undisturbed ponds at the University of Mississippi Field Station (UMFS), Abbeville, MS. Likewise, T. latifolia and M. aquaticum were collected from ponds at the UMFS. All remaining plants were collected from stock ponds at the USDA Natural Resources Conservation Service Plant Materials Center in Coffeeville, MS. Three replicate mesocosms were used for each plant species, in addition to non-vegetated sediment controls, for a total of 21 mesocosms. All mesocosms were randomly arranged, and plants were allowed six weeks to equilibrate within mesocosms prior to test initiation.

2.1. Simulated runoff

Nutrient stocks (10,000 mg L⁻¹) for nitrate, ammonium, and

orthophosphate were prepared prior to each of the two summer experiments by using reagent grade (Fisher Scientific) sodium nitrate (60.69 g L $^{-1}$), ammonium sulfate (47.17 g L $^{-1}$), and potassium phosphate dibasic (56.26 g L $^{-1}$), respectively, dissolved in 1 L of deionized water. Mixing chambers were prepared with a calculated volume of well water (1.139 \pm 0.022 mg L $^{-1}$ nitrate; 0.025 \pm 0.005 mg L $^{-1}$ ammonium; 0.068 \pm 0.008 mg L $^{-1}$ soluble reactive phosphorus) and nutrient stocks to reach a target concentration of 5 mg L $^{-1}$ (for all nutrient constituents). Nutrient exposure was constant for 4 h. Prior to exposure, water depth in each mesocosm was reduced to 1/2 of the original volume to simulate hydraulic effects of low-grade weirs commonly used in Mississippi Delta drainage systems (Kröger et al., 2008).

Nutrient-enriched water was pumped into individual mesocosms using Fluid Metering Inc. (FMITM) piston pumps, models QD-1 and QD-2 connected with 0.95 cm (o.d.) x 0.64 cm (i.d.) vinyl tubing to simulate a storm runoff event. Water travelled through each mesocosm, exiting at the surface through a discharge hose (0.95 cm \times 0.64 cm) at the opposite end of the mesocosm. Pump flow rates were adjusted so that all mesocosms maintained a 4 h hydraulic retention time (HRT). Mesocosms were exposed to flowing nutrient enriched water for 4 h, then exposed to flowing unamended well water for an additional 4 h to simulate potential flushing effects of a second storm event.

2.2. Sample collection and analysis

In the first year's experiment, water samples were collected in 237 mL HDPE containers prior to nutrient exposure (background) and hourly for 10 h following exposure from an outflow hose at the opposite end from the inflow. The following year, water samples were collected in identical containers at background, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 24, 48, 72 and 168 h post-nutrient exposure. When water was not being pumped through mesocosms, samples were collected by dipping containers at the water surface by the outflow hose. All water samples were analyzed for nitrate, ammonium, and soluble reactive phosphorus using a ThermoSpectronic (Rochester, NY, USA) Genesys 10 ultraviolet (UV) spectrophotometer. The cadmium reduction method was used to determine nitrate, whereas the standard phenate method was used to determine ammonium (APHA, 2005). Soluble reactive phosphorus (filtered orthophosphate) was determined according to the methods of Murphy and Riley (1962). All analyses were performed following filtration using a 0.45 µm cellulose membrane and were completed within 48 h of sample collection. Limits of detection were 0.001 mg L⁻¹ for soluble reactive phosphorus at 880 nm and 0.005 mg L^{-1} for nitrate and ammonium at 530 nm in a 50 mm flow

Influent nutrient loads were calculated by multiplying the inflow concentration ($\mu g L^{-1}$) by the FMITM pump rate for each mesocosm during the given time. Effluent loads were estimated by averaging outflow concentrations by sequential time steps and multiplying the mean concentration by the amount of water exiting each tub over associated time periods, which was assumed to be equal to the inflow pump rate, since a constant water level was maintained through the experiment. Percent decrease in nutrient loads exiting mesocosms after the 4 h simulated runoff, percent of nutrient load released from mesocosms during the 4 h clean water flush, and total percentage decrease in nutrient loads exiting mesocosms were calculated from the total influent loads and amount of each nutrient in the effluent over the given time frames. Significant differences in effluent nutrient loads between treatments were determined with JMP 8.0 software using analysis of variance (ANOVA) at an alpha of 0.05. A Tukey's HSD was performed to determine levels of differences between individual treatments.

3. Results

3.1. Year one

A significant difference ($F_{6,14}=2.3184$; p=0.0358) existed between the inflow mass of T. dealbata and T. latifolia. Percent mean (\pm SE) nitrate load decreases after the initial 4 h runoff ranged from $78\pm1.3\%$ (T. latifolia) to $92\pm1.2\%$ (P. hemitomon) (Table 1.) Following the 4 h unamended water flushing event, percent nitrate loads released in outflows ranged from $32\pm1.1\%$ (P. hemitomon) to $47\pm2.1\%$ (P. cernuus). Total percentage load decreases among the different plant species ranged from $32\pm2.0\%$ (P. latifolia) to $90\pm1.1\%$ (P. hemitomon) (Table 1). Panicum was significantly more efficient (P_{6,14} = 4.6025; P = 0.0088) than the control, P. latifolia, and P. cernuus in overall percent mass retention (Tukeys HSD, P < 0.05).

Panicum hemitomon, E. cordifolius, M. aquaticum, and T. dealbata were each significantly more efficient at ammonium retention after the initial 4 h runoff than unvegetated controls ($F_{6,14}=6.424$; p=0.0038). Percent mean (\pm SE) ammonium load decreases during this initial 4 h ranged from $66\pm4.6\%$ (S. cernuus) to $92\pm1.4\%$ (P. hemitomon) (Table 2). Overall total percentage load decreases among the different plant species ranged from $-2.8\pm9.6\%$ (S. cernuus) to $54\pm1.5\%$ (P. hemitomon) (Table 2). Both P. hemitomon and E. cordifolius were significantly more efficient at overall total ammonium retention than were unvegetated controls ($F_{6,14}=3.5177$; p=0.0246).

Five of the six plant species (*P. hemitomon, E. cordifolius, M. aquaticum, T. dealbata*, and *T. latifolia*) were significantly more efficient at soluble reactive phosphorus retention than unvegetated controls during the first 4 h of runoff ($F_{6,14} = 7.6561$; p = 0.0009) (Table 3). While the unvegetated control and *S. cernuus* had net increases in total percent soluble reactive phosphorus mass retention (-25 ± 2.4 and -13 ± 6.5 , respectively), *P. hemitomon, T. latifolia, E. cordifolius, M. aquaticum*, and *T. dealbata* were significantly more efficient at soluble reactive phosphorus total percent retention than the unvegetated control ($F_{6,14} = 3.5392$; p = 0.0241).

3.2. Year two

Typha latifolia inflow nitrate load (3817 \pm 357 mg) was significantly different from the unvegetated control (5860 \pm 874 mg) (F_{6,14} = 1.3692; p = 0.0379). Percent mean (\pm SE) nitrate load decreases during the initial 4 h exposure ranged from 41 \pm 12% (*T. dealbata*) to 67 \pm 7.1% (*M. aquaticum*) (Table 4). No significant differences were noted in nitrate mitigation during the 4 h exposure, subsequent 4 h flush, or in total overall nitrate decrease between vegetated systems and unvegetated controls, nor were there significant differences between different types of vegetation for these time periods.

Myriophyllum aquaticum and T. latifolia were significantly more efficient than unvegetated controls at mitigating ammonium loads during the first 4 h of exposure ($F_{6,14}=4.9402$; p=0.0065). Additionally, both M. aquaticum and T. latifolia were significantly more efficient than T. dealbata at mitigating ammonium loads during the first 4 h of exposure. Only M. aquaticum ($F_{6,14}=2.9184$; p=0.0463) lost significantly less ammonium than unvegetated controls during the 4 h unamended flush. Percentage of ammonium losses during the 4 h flush ranged from $54\pm5.6\%$ (M. aquaticum) to $82\pm4.5\%$ (S. cernuus) (Table 5).

Both *T. latifolia* and *M. aquaticum* were significantly more efficient than the unvegetated controls at mitigating soluble reactive phosphorus loads during the first 4 h of exposure ($F_{6,14} = 4.2809$; p = 0.0117) (Table 6). Percentage of soluble reactive phosphorus load retention after the first 4 h exposure ranged from $19 \pm 7.0\%$ (unvegetated control) to $58 \pm 3.4\%$ (*T. latifolia*). No significant differences existed between individual species and the unvegetated controls, nor among individual species, with respect to efficiency after the 4 h unamended flush (Table 6).

3.3. Comparison between two years

All nitrate inflow loads in year two were significantly greater than those from year one ($F_{13,28}=31.8363$; p < 0.0001). Significant decreases in percent nitrate mass retention after the initial 4 h exposure between the two years were noted for *T. dealbata*, *S. cernuus*, and *P. hemitomon* ($F_{13,28}=7.4158$; p < 0.001). Likewise, overall percent nitrate mass retention showed significant decreases between years in *T. dealbata* and *S. cernuus* ($F_{13,28}=5.4467$; p < 0.0001).

Only *S. cernuus* indicated significant differences between years in ammonium inflow mass ($F_{13,28} = 5.8594$; p < 0.0001). *Panicum hemitomon, T. dealbata, E. cordifolius*, unvegetated control, and *S. cernuus* showed significant differences (decreases) between years in percent ammonium retention after the first 4 h of exposure ($F_{13,28} = 15.8667$; p < 0.0001). *Panicum hemitomon, T. dealbata*, and *E. cordifolius* demonstrated significant differences in overall percent ammonium mass retention between the two study years ($F_{13,28} = 9.7995$; p < 0.0001). *Panicum hemitomon* annual overall ammonium mass retentions went from $54 \pm 1.5\%$ in the first year to $-23 \pm 4.9\%$ in the second year. *Thalia dealbata* and *E. cordifolius* showed similar trends, going from $31 \pm 5.2\%$ to $-42 \pm 7.4\%$ and $50 \pm 17\%$ to $-21 \pm 1.5\%$, respectively (Tables 2 and 5).

Although no significant differences in soluble reactive phosphorus mass loadings occurred between the two study years, *E. cordifolius*, *P. hemitomon*, *T. dealbata*, and the unvegetated control showed significant differences in percentage mass retention of soluble reactive phosphorus after the first 4 h of exposure $(F_{13,28} = 14.9023; p < 0.0001)$.

Table 1Year 1 mean loads (mg) and percent decrease of loads of nitrate entering and exiting mesocosms (±SE) (n = 3). Different letters indicate statistical significance among different species for each parameter.

	Typha latifolia	Panicum hemitomon	Thalia dealbata	Echinodorus cordifolus	Myriophyllum aquaticum	Saururus cernuus	Unvegetated
Total inflow (mg)	242 ±(23.5)	432 (±22.8)	562 (±147)	426 (±32.6)	429 (±45.4)	467 (±27.3)	395 (±31.6)
	a	ab	b	ab	ab	ab	ab
0-4 h outflow (mg)	53.3 (±3.19)	35.3 (±6.87)	$74.1 (\pm 19.1)$	48.8 (±7.62)	57.4 (±12.7)	$84.1 (\pm 14.0)$	70.0 (16.3)
5-8 h flush outflow (mg)	111 (±14.6)	139 (±2.94)	233 (±72.1)	184 (±8.33)	175 (±16.5)	222 (±23.1)	155 (15.0)
Total outflow (mg)	165 (±16.5)	174 (±9.78)	307 (±91.2)	233 (±15.0)	233 (±25.9)	306 (±36.7)	225 (±30.3)
% decrease after 4 h	77.8 (± 1.34)	91.9 (±1.18)	86.8 (±0.12)	88.7 (±0.89)	86.1 (±3.67)	82.1 (±2.00)	82.2 (±3.78)
	ac	b	ab	b	ab	ab	ab
% decrease after 4 h flush	$54.2 (\pm 2.84)$	67.7 (±1.14)	59.5 (±1.79)	56.7 (±1.77)	58.7 (±3.97)	52.7 (±2.10)	$60.7 (\pm 2.41)$
	bc	a	ab	ab	ab	b	ac
Total % decrease	$32.0 (\pm 1.95)$	59.6 (±1.05)	46.3 (±1.73)	45.3 (±0.94)	44.8 (±7.61)	34.9 (±4.05)	43.0 (±6.18)
	ac	b	abc	abc	abc	ac	ac

 Table 2

 Year 1 mean loads and percent decrease of loads of ammonium entering and exiting mesocosms (\pm SE) (n=3). Different letters indicate statistical significance among different species for each parameter.

	Typha latifolia	Panicum hemitomon	Thalia dealbata	Echinodorus cordifolus	Myriophyllum aquaticum	Saururus cernuus	Unvegetated
Total inflow (mg)	14.1 (±1.38)	22.7 (±1.62)	30.2 (±10.9)	21.0 (±1.73)	20.2 (±3.77)	24.1 (±3.36)	19.8 (±2.87)
0-4 h outflow (mg)	4.16 (±0.26)	$1.88 (\pm 0.44)$	5.01 (±1.34)	2.16 (±1.12)	2.91 (±1.32)	7.92 (±0.73)	$6.00 (\pm 0.64)$
	ab	b	ab	bc	bc	a	a
5-8 h flush outflow (mg)	$7.53 (\pm 1.28)$	$8.42 (\pm 0.12)$	14.6 (±4.06)	8.94 (±3.01)	9.01 (±1.83)	16.4 (±1.75)	13.1 (±1.74)
Total outflow (mg)	11.7 (±1.53)	$10.3\ (\pm0.39)$	19.6 (±5.94)	11.1 (±4.09)	11.9 (±3.14)	24.3 (±2.40)	19.1 (±2.38)
% decrease after 4 h	70.2 (±2.58)	91.9 (±1.37)	82.3 (±1.52)	90.5 (±4.95)	83.6 (±7.72)	66.2 (±4.62)	69.1 (±2.25)
	ac	b	bc	b	bc	ac	a
% decrease after 4 h flush	46.9 (±7.66)	62.5 (±2.64)	49.0 (±3.77)	59.4 (±11.9)	51.4 (±13.4)	31.0 (±4.97)	33.2 (±4.81)
	ab	b	ab	b	ab	ab	a
Total % decrease	17.1 (±9.31)	54.3 (±1.50)	31.4 (±5.15)	49.9 (±16.8)	35.0 (±21.1)	$-2.84 (\pm 9.59)$	2.31 (±6.91)
	abc	c	abc	bc	abc	ab	a

 Table 3

 Year 1 mean loads and percent decrease of loads of soluble reactive phosphorus entering and exiting mesocosms (\pm SE) (n = 3). Different letters indicate statistical significance among different species for each parameter.

	Typha latifolia	Panicum hemitomon	Thalia dealbata	Echinodorus cordifolus	Myriophyllum aquaticum	Saururus cernuus	Unvegetated
Total inflow (mg)	421 (±52.0)	511 (±25.2)	715 (±140)	603 (±45.4)	582 (±13.7)	661 (±42.3)	543 (±77.1)
0-4 h outflow (mg)	136 (±14.6)	76.2 (±22.9)	203 (±61.5)	126 (±21.8)	146 (±37.8)	264 (±36.0)	253 (±39.6)
	bc	c	abc	bc	abc	ab	a
5-8 h flush outflow (mg)	230 (±36.7)	293 (±6.57)	518 (±166)	404 (±32.2)	395 (±25.1)	483 (±42.8)	425 (±51.5)
Total outflow (mg)	$366 (\pm 48.0)$	369 (±29.2)	721 (±227)	530 (±51.9)	540.7 (±60.5)	746 (±75.9)	$678 (\pm 90.9)$
% decrease after 4 h	67.4 (±2.25)	$85.4 (\pm 3.60)$	$72.8 (\pm 2.92)$	78.6 (±4.87)	74.9 (±6.28)	60.1 (±4.21)	$53.6 (\pm 1.30)$
	bc	c	bc	bc	bc	ab	a
% decrease after 4 h flush	$44.2 (\pm 10.8)$	42.6 (±1.50)	30.8 (±8.39)	32.3 (±7.69)	32.3 (±3.36)	27.2 (±2.45)	21.3 (±2.20)
	b	b	ab	ab	ab	ab	a
Total % decrease	11.5 (±13.1)	28.1 (±2.14)	3.55 (±11.3)	10.85 (±12.6)	7.26 (±9.53)	$-12.7 (\pm 6.51)$	$-25.2 (\pm 2.44)$
	b	b	b	b	b	ab	a

Table 4Year 2 mean loads and percent decrease of loads of nitrate entering and exiting mesocosms (\pm SE) (n = 3). Different letters indicate statistical significance among different species for each parameter.

	Typha latifolia	Panicum hemitomon	Thalia dealbata	Echinodorus cordifolus	Myriophyllum aquaticum	Saururus cernuus	Unvegetated
Total inflow (mg)	3817 (±357) b	4817 (±460) ab	4889 (±518) ab	5537 (±505) ab	5817 (±567) ab	5620 (±912) ab	5860 (±874)
0-4 h outflow (mg)	1273 (±137)	2082 (±177)	2994 (±915)	2393 (±146)	1966 (±615)	3084 (±415)	2539 (±619)
5-8 h flush outflow (mg)	2181 (±299)	3014 (±221)	4386 (±1591)	3705 (±116)	2632 (±258)	4582 (±436)	3496 (±1020)
Total outflow (mg)	3455 (±435)	5096 (±302)	7380 (±2505)	6098 (±262)	4597 (±764)	7666 (±573)	6035 (±1638)
% decrease after 4 h	66.5 (±2.22)	56.1 (±4.88)	40.8 (±12.1)	55.7 (±6.18)	67.4 (±7.08)	41.0 (±15.9)	58.1 (±5.23)
% decrease after 4 h flush	43.1 (±3.81)	37.1 (±1.78)	13.9 (±22.7)	31.7 (±7.80)	53.9 (±6.66)	12.2 (±19.3)	43.3 (±10.3)
Total % decrease	9.62 (±5.81)	$-6.71 (\pm 5.53)$	$-45.3~(\pm 34.8)$	$-12.7~(\pm 14.0)$	21.3 (±8.28)	$-46.8 \ (\pm 33.2)$	1.41 (±15.5)

 Table 5

 Year 2 mean loads and percent decrease of loads of ammonium entering and exiting mesocosms (\pm SE) (n=3). Different letters indicate statistical significance among different species for each parameter.

	Typha latifolia	Panicum hemitomon	Thalia dealbata	Echinodorus cordifolus	Myriophyllum aquaticum	Saururus cernuus	Unvegetated
Total inflow (mg)	34.9 (±3.27)	42.3 (±2.45)	49.7 (±14.9)	49.7 (±2.13)	45.3 (±2.36)	55.3 (±1.49)	48.3 (±7.54)
0-4 h outflow (mg)	$12.4 (\pm 1.10)$	23.2 (±3.20)	31.8 (±10.2)	24.2 (±3.23)	16.0 (±5.45)	32.4 (±1.35)	28.2 (±5.54)
	b	ab	ab	ab	ab	ab	a
5–8 h flush outflow (mg)	19.9 (±2.58)	28.7 (±0.71)	39.3 (±12.5)	36.2 (±0.94)	24.8 (±3.39)	45.4 (±1.43)	37.5 (±9.84)
Total outflow (mg)	32.3 (±3.68)	51.9 (±3.55)	71.0 (±22.7)	60.4 (±3.17)	40.8 (±7.31)	77.8 (±2.45)	65.6 (±15.2)
	b	ab	ab	ab	ab	ab	a
% decrease after 4 h	64.3 (±2.98)	45.5 (±5.14)	36.7 (±1.81)	51.7 (±4.91)	65.5 (±10.2)	41.4 (±2.68)	42.3 (±3.12)
	b	abc	c	abc	b	abc	ac
% decrease after 4 h flush	42.6 (±6.25)	31.6 (±4.50)	21.0 (±5.82)	26.9 (±3.37)	45.6 (±5.57)	17.7 (±4.48)	25.7 (±10.5)
	ab	ab	ab	ab	b	ab	a
Total % decrease	6.89 (±9.14)	$-22.9(\pm 4.91)$	$-42.4~(\pm 7.44)$	$-21.4(\pm 1.54)$	11.0 (±11.5)	$-40.9 (\pm 6.72)$	-32.0(12.5)
	b	abcd	cd	abcd	b	d	acd

4. Discussion

Most of the applied literature examines mitigation efficiency of plant mixtures or wetlands as a whole, rather than focusing on individual plant species success (or failure). The current line of research sought to identify successful individual species before evaluating them as part of mixtures. Of the six plant species examined, *P. hemitomon* was most effective at mitigation of nitrate,

Table 6 Year 2 mean loads and percent decrease of loads of soluble reactive phosphorus entering and exiting mesocosms (\pm SE) (n = 3). Different letters indicate statistical significance among different species for each parameter.

	Typha latifolia	Panicum hemitomon	Thalia dealbata	Echinodorus cordifolus	Myriophyllum aquaticum	Saururus cernuus	Unvegetated
Total inflow (mg)	612 (±60.2)	775 (±68.6)	1053 (±385)	854 (±25.5)	871 (±97.8)	1093 (±55.9)	822 (±82.7)
0—4 h outflow (mg)	264 (±45.1)	520 (±96.9)	753 (±241)	623 (±72.6)	428 (±128)	707 (±23.9)	670 (±102)
	b	ab	ab	ab	ab	ab	a
5-8 h flush outflow (mg)	451 (±88.5)	735 (±19.6)	740 (±274)	633 (±95.6)	693 (±94.8)	866 (±90.5)	731 (±123)
Total outflow (mg)	715 (±134)	1256 (±98.0)	1493 (±510)	1256 (±161)	1121 (±196)	1572 (±93.3)	1401 (±218)
% decrease after 4 h	57.6 (±3.41)	$34.0 (\pm 6.88)$	26.3 (±6.92)	$27.0 (\pm 8.78)$	52.8 (±8.50)	34.9 (±5.0)	19.1 (±7.03)
	b	ab	ab	ab	b	ab	a
% decrease after 4 h flush	27.9 (±7.99)	3.71 (±8.45)	28.6 (±18.7)	25.5 (±12.4)	19.4 (±12.1)	19.7 (±12.2)	11.1 (±12.0)
Total % decrease	$-14.6 (\pm 11.4)$	$-62.3 (\pm 2.05)$	$-45.0 (\pm 25.3)$	$-47.5 (\pm 20.6)$	$-27.8 (\pm 13.6)$	$-45.4 (\pm 16.0)$	$-70 (\pm 18.5)$
	b	ab	ab	ab	ab	ab	a

ammonium, and soluble reactive phosphorus during the first year. *Panicum hemitomon* is often used for shoreline stabilization due to its rapid, dense growth rate. Additionally, it dissipates wave energy and traps suspended sediment (Shadow, 2004). While absent in brackish water environments, *P. hemitomon* is cold tolerant down to –19 °C (Shadow, 2004). These attractive benefits must also be weighed against the plant's ability to outcompete some native grasses before using it in mitigation strategies. Likewise during the first year, *S. cernuus* and *T. latifolia* were the least effective at mitigating nitrate and ammonia, while the unvegetated control was least effective for soluble reactive phosphorus mitigation.

In the second year, however, *M. aquaticum* was the most effective plant species examined for mitigating nitrate and ammonium, while *T. latifolia*, and to some degree *T. dealbata*, was most effective for soluble reactive phosphorus mitigation. *Saururus cernuus* and *T. dealbata* were the least effective plants at mitigating nitrate and ammonium in the second year. The unvegetated control was once again the least effective among treatments at mitigating soluble reactive phosphorus during the second year. Changes among years in species efficiency were likely influenced by the significantly higher exposure loads of nutrients in the second year as opposed to the first year.

As stated earlier, many comparable studies examine nutrient mitigation efficiency of landscape systems, such as wetlands, rather than individual plant species. Tournebize et al. (2015) reported a $50 \pm 18\%$ decrease in nitrate flux at the outflow of constructed wetlands in France, while Groh et al. (2015) saw similar results (56% decrease in total nitrate load) over a two-year examination of wetlands in east-central Illinois. While observing a 39% decrease in nitrate concentration from inflow to outflow, Brauer et al. (2015) determined a 75% nitrate removal efficiency (based on water and nitrate mass balance) in a restored wetland in the San Joaquin River valley of California.

In an earlier study, Moore and Kröger (2011) assessed the same six aquatic plant species as the current study; however, while their nutrient exposure was also 4 h, it was followed by an 8 h flush of unamended water as opposed to the 4 h in the current study. Results from their study indicated E. cordifolius decreased nitrate loads by $63 \pm 18\%$ after 4 h, while in the current study, E. cordifolius decreased nitrate loads by $89 \pm 0.89\%$ after 4 h (Moore and Kröger, 2011). Each of the individual species in the first year of the current study had better nitrate retention than in the Moore and Kröger (2011) study. Additionally the current total percent decrease in nitrate loads was much greater than those reported in Moore and Kröger (2011). Current ammonium load decreases compared to results from Moore and Kröger (2011) mirrored the above example of nitrate load decreases, where the current study demonstrated much greater efficiency, even though P. hemitomon was the most successful plant species with regard to ammonium load mitigation in the both studies. This demonstrates the variability often reported

in studies examining phytoremediation of nutrients (Deaver et al., 2005; Moore et al., 2013).

Although some studies suggest nutrient retention is more the result of other processes instead of vegetation (Huttunen et al., 1996), several studies have confirmed vegetation's importance in the retention process (García-Lledó et al., 2011). Because they can create aerobic microsites around their roots, certain macrophytes provide suitable sites for coupled nitrification and denitrification processes. Kreiling et al. (2011) found macrophytes in a backwater lake environment assimilated 29.5 mg N m $^{-2}$ d $^{-1}$, double that of sediment microbes (14.4 mg N m $^{-2}$ d $^{-1}$). Although denitrification accounted for more than 80% of the nitrate loss, assimilation was still a valuable contribution to the mitigation. While denitrification rates plateaued at nitrate concentrations of 5 mg N L^{-1} , assimilation rates generally increased with increasing concentration (Kreiling et al., 2011). In a two-season study, Borin and Salvato (2012) found Typha removed 96% and 97% of the cumulative total nitrogen load, while allocating 51-83% of nitrogen in aerial tissues.

Dissolved or soluble reactive phosphorus is the predominant form of phosphorus found in agricultural runoff (Buda et al., 2009). It is this same form of phosphorus which is available to support algal growth, helping to contribute to the hypoxia issue in the Gulf of Mexico (Lee and Jones-Lee, 2004). Constructed wetlands may be less effective at long-term reduction of phosphorus since substrate binding sites rapidly become saturated (White et al., 2011). However, several studies have documented the success of plant-based mitigation of phosphorus runoff. Moustafa et al. (2012) found that wetland treatments lacking emergent vegetation generated the most particulate phosphorus, regardless of whether the phosphorus loading rate was low or high. A three-year mesocosm study with the Everglades' stormwater treatment area vegetation reported a 51% decrease in average outflow phosphorus concentrations, while a two-year time frame was needed before the local Florida soils became a phosphorus sink (Mitsch et al., 2015).

Torit et al. (2012) studied uptake of phosphorus by E. cordifolius, reporting it was able to remove 16% of phosphorus from domestic wastewater, more than twice that capable of being removed by microorganisms in water (7%) or microorganisms in soil (6%). Although adsorption by soil was still the major source of phosphorus removal in the study (71%), E. cordifolius demonstrated a high phosphorus uptake efficiency (approximately one day) (Torit et al., 2012). Thalia dealbata was capable of removing phosphorus in the system, but did not grow well in the domestic wastewater source (Torit et al., 2012). In a previous study examining the same six plant species as the current study, Moore and Kröger (2011) reported peak soluble reactive phosphorus decreases ranging from 40 to 59%, while the current study demonstrated decreases ranging from 54 to 85%. In both studies, T. latifolia, S. cernuus, and the unvegetated control, showed the least effectiveness at decreasing soluble reactive phosphorus during the initial 4 h exposure. Similarly, total percent soluble reactive phosphorus load decreases ranged from -61 to 4% in Moore and Kröger (2011), and from -25 to 28% in the current study.

5. Conclusions

Millions of dollars of Federal and private funds have been invested in researching ways to reduce nutrient transport to the Gulf of Mexico. Even with this investment, there still remains an urgent need to perform quantitative assessments of land and crop nutrient application management practices, as well as edge-of-field practices, to address this issue (Lee and Jones-Lee, 2004). The complexity of these issues is compounded by the public demand for improvement. Even the National Research Council (2009) acknowledged the difficulty in tracking downstream impacts from upstream pollutants in the Mississippi River Basin, noting the need for years of monitoring and assessment. Scientists must continue to address the problem both directly on the field through nutrient management and at the edge of the field reducing impacts of nutrient loss. The current study provides critical species-specific nitrogen and phosphorus mitigation efficiency in a small-scale multi-year setting. Results indicate the importance of multispecies edge-of-field vegetated systems to address sustainable mitigation of nutrient runoff, as well as multi-year assessments to further understand the inherent variability associated with nutrient phytoremediation. Future studies should examine mixed populations of native aquatic vegetation for their efficiency in nutrient mitigation.

Acknowledgments

Thanks to Lisa Brooks and Wood Dabbs for sample collection assistance, preparation, and analyses. The USDA is an equal opportunity employer. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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