Runoff pH Influences Nutrient Removal Efficacy of Floating Treatment Wetland Systems

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SUMMARY. Floating treatment wetlands (FTWs), a modified constructed wetland technology, can be deployed in ponds for the treatment of nursery and greenhouse irrigation runoff. The pH of nursery and greenhouse operation irrigation water varies from 3.3 to 10.4 across the United States. Water flow rate, plant species selection, and variable nutrient inputs influence the remediation efficacy of FTWs and may interact with the pH of inflow water to change nutrient remediation dynamics. Therefore, an experiment was designed to quantify the effect of pH on the growth and nutrient uptake capacity of three macrophyte species using a mesocosm FTW system. ‘Rising Sun’ japanese iris (*Iris ensata*), bushy bluestem (*Andropogon glomeratus*), and maidencane (*Panicum hemitomon*) were grown for two 6-week periods and exposed to five pH treatment levels representing the range of nursery and greenhouse irrigation runoff, 4.5, 5.5, 6.5, 7.2, and 8.5, for a total of 15 plant and pH combinations. Water was treated with either hydrochloric acid to decrease the pH or sodium hydroxide to increase the pH. The pH-adjusted solutions were mixed with 12 mg·L⁻¹ nitrogen (N) and 6 mg·L⁻¹ phosphorus (P) fertilizer (64.8 g·m⁻³ N and 32.4 g·m⁻³ P). Differences in pH impacted both N and P removal from the FTW systems for two of the three species studied, maidencane and bushy bluestem. Higher pH treatments reduced nutrient removal efficacy, but plants were still capable of consistently removing nutrients across all pH treatments. Conversely, ‘Rising Sun’ japanese iris maintained similar remediation efficacies and removal rates across all pH treatments for both N and P, possibly due to the ability to acidify its rhizosphere and modify the pH of the system. Average N and P loads were reduced by 47.3 g·m⁻³ N (70%) and 16.6 g·m⁻³ P (56%). ‘Rising Sun’ japanese iris is a promising plant for use in highly variable conditions when the pH of irrigation runoff is outside the typical range (5.5–7.5). Results from model simulations poorly predict the nutrient availability of P and ammonium in effluent, most likely due to the inability to determine plant and biological contributions to the system, such as N-fixing bacteria.

Nursery and greenhouse crop production often results in high concentrations of nutrients within production runoff. Effluent nutrient concentrations can range from 0.1 to 387 mg·L⁻¹ nitrate-nitrogen (NO₃-N), 0.9 to 47 mg·L⁻¹ ammoniacal-nitrogen (NH₄-N), and 0.01 to 306 mg·L⁻¹ total P (Dole et al., 1994; Prystay and Lo, 2001; Roseth and Haarstad, 2010; White, 2013; Wilson et al., 2010). FTWs effectively remediate both N and P using a buoyant floating surface planted with macrophyte species (Tanner and Headley, 2011; White and Cousins, 2013). In a review of FTW systems, Pavlineri et al. (2017) identified more than 42 FTW experiments to date evaluating FTW performance for a variety of parameters. The pH at which the majority of those experiments were conducted was neutral or near neutral,

between 6.2 and 7.4. However, the pH of nursery and greenhouse runoff is much more variable.

Argo et al. (1997) conducted a geographical analysis of irrigation water applied to greenhouse operations

across the United States and Canada and found that the pH of water applied as irrigation ranged from 2.7 to 11.3, and that the alkalinity of water applied as irrigation ranged from 0 to 1120 mg·L⁻¹ calcium carbonate (CaCO₃). The overall mean pH of all water samples was 7.0, with a median value of 7.1. Of the samples, 44% had a pH between 5 and 7, but 53% had a pH >7 (Argo et al., 1997). Although the pH of applied irrigation water does not directly translate to the pH of greenhouse or nursery runoff, few studies have characterized the quality of irrigation runoff on a nationwide basis. Chen et al. (2003) reported that runoff water had a higher pH than irrigation water (7.6 in well water vs. 9.7 in captured water). Changes in pH largely depend on geography, planting substrate and amendments, and irrigation system design, among other factors, including alkalinity. Alkalinity is a measure of the buffering capacity of water; when it is high, it can increase pH (Kuehny and Morales, 1998).

For the growth of greenhouse crops using a soilless substrate, the general consensus is that the ideal pH range is 5 to 7 (Argo et al., 1997; Chen et al., 2003). However, assessments of plant growth in aquatic systems, such as the conditions for plants grown in FTWs, are lacking. Research related to pH and crop growth in hydroponic or aquaponic systems may be most closely aligned with conditions in FTWs. Microbial nitrification of NH₄⁺ to nitrite (NO₂⁻) and NO₂⁻ to NO₃⁻ is optimized at pH 8.5. Plant nutrient uptake for many crop species is optimized with a pH near 6.0; therefore, the pH in aquaponic systems is

Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.0929	ft ²	m ²	10.7639
0.0283	ft ³	m ³	35.3147
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	micron(s)	µm	1
28.3495	oz	g	0.0353
305.1517	oz/ft ²	g·m ⁻²	0.0033
7.4892	oz/gal	g·L ⁻¹	0.1335
37.0798	oz/yd ³	g·m ⁻³	0.0270
1	ppm	mg·L ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

managed near 7.0 (Wortman, 2015). Zou et al. (2016) determined that a pH of 6.0 was optimal for plant growth and N utilization efficiency in aquaponics, but it resulted in increased nitrous oxide (N_2O) emissions due to high denitrification. Solution pH further impacts P availability and the forms in which P exists. Dissociation of phosphoric acid (H_3PO_4) to dihydrogen phosphate (H_2PO_4^-) and then to hydrogen phosphate (HPO_4^{2-}) occurs at pH 2.1 and 7.2, respectively (Schachtman et al., 1998). Plants can only absorb P as the free orthophosphate ions H_2PO_4^- and HPO_4^{2-} (Becquer et al., 2014). Therefore, the rate of P uptake is directly related to the pH of the solution (White, 2012). Knowledge of pH effects is important for managing nutrient remediation and uptake by FTW systems treating runoff from greenhouse and nursery operations.

Previous research in a variety of disciplines (forest ecology, wetland ecology, hydroponics, etc.) has suggested that plant growth and nutrient uptake vary by species, cultivar, and

the characteristics of the system (Härdtle et al., 2004; Wagner et al., 2016; Wortman, 2015). Furthermore, some plant species directly influence their growing conditions through root-induced pH changes. These changes of pH in the rhizosphere are a long-documented chemical interaction, but they mostly result from root-soil interactions. Roots can substantially alter their rhizosphere pH by releasing hydrogen (H^+) or hydroxide (OH^-) ions, cation-anion exchange balance, organic anion release, root exudation and respiration, and redox-coupled processes (Hinsinger et al., 2003). Numerous authors have shown that the processes by which rhizosphere change occurs depend largely on nutritional limitations within the environment (Bertrand et al., 1999; Grinstead et al., 1982; Imas et al., 1997; Neumann and Römhild, 1999).

This study was conducted to determine how pH impacts the N and P remediation efficacies of three species of plants and to identify any root-induced pH changes by the three different species of plants. Visual MINTEQ 3.1, an equilibrium-based computer model for the calculation of chemical speciation and solubility of dissolved mineral phases in aqueous solution (Gustafsson, 2012), was used to simulate the speciation and activity of key nutrients in an aqueous solution as a function of pH.

Materials and methods

EXPERIMENTAL SETUP. The experiment was repeated using two

6-week studies (22 Apr.–1 June 2016 and 17 Mar.–28 Apr. 2017). An experimental system comprising 50 10-gal plastic tubs (Rough and Rugged; United Solutions, Leominster, MA) arranged in a completely randomized design was assembled in Pendleton, SC (Fig. 1). Each tub or experimental unit (EU) had a surface area of 0.17 m^2 and a volume of 0.07 m^3 . The experimental setup was located inside a greenhouse to maintain environmental control and exclude rainfall. Five 110-gal tanks (Vertical Water Storage; Poly-Mart, Austin, TX) were outfitted with PVC lines and served as holding tanks for each pH treatment. Water pH in each holding tank was adjusted using a handheld multimeter (Professional Plus; YSI, Yellow Springs, OH) calibrated to the appropriate treatment level, as selected based on nursery runoff ranges of 4.5, 5.5, 6.5, 7.2 (baseline), and 8.5, and thoroughly mixed before filling individual EUs. At the bottom of each holding tank, a water hose connected with a pump was installed to allow filling of the EUs.

Two-centimeter-thick floating mats (Beemats, New Smyrna Beach, FL) were cut into three 10×10 -cm squares with 7.5-cm pre-cut holes located in the center of the cut mat for each EU. The holes allowed insertion of specially designed aerator cups in which plants were placed. Three species of plants were used in this study, bushy bluestem, 'Rising Sun' japanese iris, and maidencane. 'Rising Sun' japanese iris were supplied as rhizomes by Terra Ceia Farms

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Fig. 1. Experimental setup for floating wetland experiments including 50 experimental units and five holding tanks for each pH (4.5, 5.5, 6.5, 7.2, and 8.5) and demonstration of the 10×10 -cm (3.9 inches) floating squares with specially designed aerator cups and plants.

Table 1. Characteristics of mineral nutrient composition of simulated specialty crop production runoff used to parameterize a Visual MINTEQ 3.1 model predicting speciation changes as influenced by solution pH of 4.5, 5.5, 6.5, 7.2, and 8.5 (Gustaffson, 2012).

Parameter	Value (mg·L ⁻¹) ^z
Aluminum	ND
Calcium	8.44
Chloride	10.43
Copper	0.087
Iron	0.30
Potassium	10.63
Magnesium	1.51
Manganese	0.03
Molybdenum	ND
Sodium	12.58
Ammonium	1.696
Nickel	ND
Nitrite	ND
Nitrate	10.65
Phosphate	5.79
Sulfate	17.99
Zinc	0.062

^zND = nondetectable and input as 0.0; 1 mg·L⁻¹ = 1 ppm.

(Pantego, NC), and bushy bluestem and maidencane were sourced as bare root liners (3–4 inches long) from Pinelands Nursery (Columbus, NJ). For each EU, three plants of one species were placed in aerator cups with 15 EUs allocated to each species (Fig. 1). Three EUs for each plant species and an additional control tub with no plants were filled with each pH solution, for a total of 50 EUs.

SIMULATION OF RUNOFF CONTAINING NUTRIENTS. Holding tanks were first filled with municipal water. Municipal water had an average pH of 7.2 and alkalinity of 13.3 mg·L⁻¹ CaCO₃, with all constituents detected below the maximum contaminant level. Water was then spiked with fertilizer to attain concentrations of 12 mg·L⁻¹ N. The simulated nursery runoff was prepared by dissolving 72.6 g·L⁻¹ of 20N-0.9P-16.6K water-soluble fertilizer (Nitrate Special Soluble Fertilizer; Southern Agricultural Insecticides, Hendersonville, NC) in each 110-gal water holding tank. Finally, water was treated with hydrochloric acid (HCl) to decrease the pH or sodium hydroxide (NaOH) to increase the pH. Target pH levels were obtained by adding

Fig. 2. Treatment pH levels for experimental units of (A) 4.5, (B) 5.5, (C) 6.5, (D) 7.2, and (E) 8.5 over a 7-d period as affected by three plant species (bushy bluestem, maidencane, and 'Rising Sun' japanese iris) compared with an unplanted control. Lines are an average (n = 6) across two experiments. Results are means ± SE.

60 mL HCl (4.5), 40 mL HCl (5.5), 20 mL HCl (6.5), nothing (7.2), or 25 mL NaOH (8.5) to each holding tank. Each EU was filled from the

holding tanks on day 0 and then drained on day 7 to simulate 7-d hydraulic retention time over the 6-week experiments.

Table 2. Ionic total nitrogen (ammonium + nitrite + nitrate) and phosphorus material balance calculations for bushy bluestem across five pH treatments (4.5, 5.5, 6.5, 7.2, 8.5) for 2 years (2016 and 2017) after 6-week exposure to nutrients in floating treatment wetlands.

	Total ionic nitrogen [mean $\pm$ SE (g·m ⁻³)] ^a									
	4.5		5.5		6.5		7.2		8.5	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Material balance										
Total influent load	61.5 (5.61)	76.3 (3.75)	62.2 (7.37)	77.6 (2.71)	66.1 (12.3)	75.2 $\pm$ 2.18	58.6 $\pm$ 6.31	74.9 $\pm$ 2.20	61.6 $\pm$ 5.24	75.5 $\pm$ 3.44
Total effluent load	11.0 $\pm$ 9.83	39.1 $\pm$ 19.4	18.2 $\pm$ 15.4	43.7 $\pm$ 16.3	24.7 $\pm$ 15.5	43.9 $\pm$ 11.6	21.9 $\pm$ 16.2	49.3 $\pm$ 10.3	27.4 $\pm$ 20.2	58.3 $\pm$ 21.1
Total load reduction	50.5	37.2	44	33.8	41.4	31.3	36.7	25.7	34.3	17.2
Load reduction (%)	82.1%	48.7%	70.7%	43.6%	62.6%	41.6%	62.6%	34.2%	55.6%	22.7%
Plant uptake	17.9	9.1	15.1	6.82	18.2	11.4	10.6	7.8	12.8	4.38
Plant uptake (%)	35.4%	24.4%	34.2%	20.2%	43.9%	36.4%	28.9%	30.4%	37.3%	25.5%
Other removal processes	32.7	28.1	29	27	23.2	19.9	26.1	17.9	21.5	12.8
Total phosphorus [mean $\pm$ SE (g·m ⁻³)]										
	4.5		5.5		6.5		7.2		8.5	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Material balance										
Total influent load	27.7 $\pm$ 1.43	32.1 $\pm$ 2.13	26.8 $\pm$ 1.35	32.2 $\pm$ 1.90	28.8 $\pm$ 2.5	31.7 $\pm$ 1.22	27.4 $\pm$ 2.28	35.2 $\pm$ 8.56	30.4 $\pm$ 5.39	32.3 $\pm$ 1.54
Total effluent load	7.35 $\pm$ 6.44	22.8 $\pm$ 6.65	10.2 $\pm$ 6.78	24.8 $\pm$ 4.88	13.6 $\pm$ 5.16	25.5 $\pm$ 3.20	11.1 $\pm$ 6.00	25.5 $\pm$ 4.07	12.2 $\pm$ 4.62	25.9 $\pm$ 4.04
Total load reduction	20.4	9.35	16.5	7.45	15.2	6.23	16.3	9.73	18.2	6.42
Load reduction (%)	73.5%	29.1%	61.8%	23.1%	52.9%	19.7%	59.5%	27.7%	60.0%	19.9%
Plant uptake	9.15	1.49	9.3	0.922	5.97	1.66	0.225	1.15	4.16	0.99
Plant uptake (%)	44.9%	15.9%	56.3%	12.3%	39.3%	26.6%	1.38%	11.8%	22.8%	15.4%
Other removal processes	11.20	7.86	7.22	6.53	9.24	4.57	16.1	8.58	14.1	5.41

^a1 g·m⁻³ = 0.0270 oz./yard³.

WATER SAMPLING AND ANALYSIS. Water samples were collected from the storage tanks during each fill for baseline water analysis. Water samples were collected on day 7 over the two 6-week experiments. Additional water samples were collected on day 3 and day 5 every 2 weeks beginning at week 2. Water samples were processed for analysis using two analytical methods: inductively coupled plasma optical emission spectroscopy (ICP-OES) and ion chromatography (IC). All ICP-OES samples were immediately transferred to vials with no filtration or acidification and placed in a -25 °C freezer. IC water samples were filtered using a 0.22- μ m Luer lock filter and then placed in a -25 °C freezer. Trace elements including P, potassium, calcium (Ca), magnesium, zinc, copper, manganese, molybdenum, nickel, iron (Fe), sulfur, sodium, boron, and aluminum were analyzed via ICP-OES (iCAP 6500; Thermo Scientific, Waltham, MA). Anions including NH₄⁺, NO₂⁻, NO₃⁻, phosphate (PO₄³⁻), and sulfate were measured using an ion chromatograph (AS10; Dionex Corp., Sunnyvale, CA) with an auto-sampler (AS50; Dionex Corp., Sunnyvale, CA). For anions, the lower detection limit was 0.2 mg·L⁻¹. All analyses were conducted according to U.S. Environmental Protection Agency (USEPA) protocol methods 6010B and 9056A, and calibration standards were instituted for quality assurance and control (USEPA, 1997, 2007). Environmental parameters, including pH, dissolved oxygen [DO (milligrams per liter)], and temperature (°C), were measured in a consistent manner using a calibrated handheld multimeter (YSI) on days 0, 3, 5, and 7 each week for 6 weeks. All samples were collected during the morning between 0700 and 0900 HR at a depth of 15 cm in each EU.

PLANT SAMPLING AND ANALYSIS. The roots (tissue below the mat) and shoots (tissue above the mat) of three plants per species were harvested before each experiment start date. At the end of the 6-week period, one plant from each experimental tub (n = 45) was harvested to quantify changes in the nutrient composition. The harvest process included measurements (in cm) of height and width in two directions of the shoots and roots,

Table 3. Ionic nitrogen (ammonium + nitrite + nitrate) and phosphorus material balance calculations for maidencane across five pH treatments (4.5, 5.5, 6.5, 7.2, 8.5) for 2 years (2016 and 2017) after 6-week exposure to nutrients in floating treatment wetlands.

	Total ionic nitrogen [mean $\pm$ SE (g·m ⁻³)] ^a									
	4.5		5.5		6.5		7.2		8.5	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Material balance										
Total influent load	61.5 $\pm$ 5.61	76.3 $\pm$ 3.75	62.2 $\pm$ 7.37	77.6 $\pm$ 2.71	66.1 $\pm$ 12.3	75.2 $\pm$ 2.18	58.6 $\pm$ 6.31	74.9 $\pm$ 2.2	61.6 $\pm$ 5.24	75.5 $\pm$ 3.44
Total effluent load	5.15 $\pm$ 7.76	32.1 $\pm$ 17.9	7.00 $\pm$ 6.98	43.1 $\pm$ 10.9	51.5 $\pm$ 18.3	55.9 $\pm$ 17.6	55.9 $\pm$ 39.6	48.2 $\pm$ 18.8	23.0 $\pm$ 17.4	63.8 $\pm$ 25.6
Total load reduction	56.4	44.2	55.2	34.4	14.6	19.4	2.68	26.8	38.6	11.6
Load reduction (%)	91.6%	58.0%	88.7%	44.4%	22.2%	25.7%	4.6%	35.7%	62.7%	15.4%
Plant uptake	24.1	12.4	20.3	3.99	5.4	5.0	6.21	8.35	11.4	5.3
Plant uptake (%)	42.8%	28.1%	36.7%	11.6%	36.6%	25.9%	232.0%	31.2%	29.5%	45.5%
Other removal processes	32.3	31.8	35	30.5	9.29	14.3	-3.53	18.4	27.3	6.4
	Total phosphorus [mean $\pm$ SE (g·m ⁻³)]									
	4.5		5.5		6.5		7.2		8.5	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Material balance										
Total influent load	27.7 $\pm$ 1.43	32.1 $\pm$ 2.13	26.8 $\pm$ 1.35	32.2 $\pm$ 1.9	28.8 $\pm$ 2.50	31.7 $\pm$ 1.22	27.4 $\pm$ 2.28	35.2 $\pm$ 8.56	30.4 $\pm$ 5.39	32.3 $\pm$ 1.54
Total effluent load	5.93 $\pm$ 3.68	21.3 $\pm$ 6.56	9.02 $\pm$ 4.28	25.8 $\pm$ 3.97	21 $\pm$ 8.72	24.6 $\pm$ 2.65	15.1 $\pm$ 7.04	24.6 $\pm$ 3.78	12.1 $\pm$ 2.87	25.8 $\pm$ 3.55
Total load reduction	21.8	10.8	17.7	6.36	7.75	7.09	12.3	10.6	18.3	6.53
Load reduction (%)	78.6%	33.7%	66.3%	19.8%	26.9%	22.4%	44.7%	30.2%	60.1%	20.2%
Plant uptake	12.4	6.83	13.4	0.443	0.17	1.00	0.84	2	9.04	5.03
Plant uptake (%)	57.1%	63.2%	75.3%	7.0%	2.1%	14.0%	6.85%	18.8%	49.5%	77.0%
Other removal processes	9.35	3.98	4.39	5.92	7.59	6.1	11.4	8.63	9.21	1.50

^a1 g·m⁻³ = 0.0270 oz./yard³.

followed by separation of the roots and shoots. Roots and shoots were weighed (grams fresh weight), dried at 80 °C, weighed (grams dry weight), and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 40-mesh screen (0.425 mm). Carbon (C) and N (total) in plant tissue were determined by flash combustion and gas chromatography separation [NC analyzer (CN soil flash EA1112; CE Elantech, Lakewood, NJ)]. Operational parameters were 900 and 850 °C for the primary column furnace and secondary column furnace, respectively. The column oven was set at 50 °C. Sample incendiary gas was oxygen at 250 mL·min⁻¹ at sample ignition with the carrier gas helium at 140 mL·min⁻¹. The instrument was standardized on 2,5-Bis (5-ter-butyl-benzoxazol-2-yl)thiophene [BBOT (6% to 7% N and 65% to 80% C)] with tomato (*Solanum lycopersicum*) leaf tissue (dried and ground) acquired from the National Institute of Standards and Technology (guaranteed elemental analysis) serving as the quality control check. Potassium, P, Ca, magnesium, zinc, copper, manganese, molybdenum, nickel, Fe, sulfur, sodium, boron, and aluminum concentrations in plant tissues were determined by ICP-OES, with calibration standards rerun at the midpoint and end of each analytical run.

VISUAL MINTEQ SIMULATIONS.

The effect of pH on nutrient availability and speciation was simulated using Visual MINTEQ 3.1 (Gustafsson, 2012), as outlined by Cerozi and Fitzsimmons (2016). Input values for the model are shown in Table 1. Input values for the Visual MINTEQ model were a base solution of the municipal water (pH = 7.2) and fertilizer additions. The initial baseline was then simulated at different pH levels (4.5, 5.5, 6.5, and 8.5), including addition of any chemicals used to adjust solution pH. Comparisons between model results (modeled) and initial (experimental) ICP-OES water samples at day 0 were assessed with preference over IC results due to the high level of filtration used (0.22 μ m) in IC sample preparation, thus removing elements present in the Visual MINTEQ model. When elements were unavailable through ICP-OES, IC results were used. Assessment of the biological impact (day 7 samples),

Table 4. Ionic nitrogen (ammonium + nitrite + nitrate) and phosphorus material balance calculations for 'Rising Sun' japanese iris across five pH treatments (4.5, 5.5, 6.5, 7.2, 8.5) for 2 years (2016 and 2017) after 6-week exposure to nutrients in floating treatment wetlands.

	Total ionic nitrogen [mean $\pm$ SE (g·m ⁻³)] ^a									
	4.5		5.5		6.5		7.2		8.5	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Material balance										
Total influent load	61.5 $\pm$ 5.61	76.3 $\pm$ 3.75	62.2 $\pm$ 7.37	77.6 $\pm$ 2.71	66.1 $\pm$ 12.3	75.2 $\pm$ 2.18	58.6 $\pm$ 6.31	74.9 $\pm$ 2.2	61.6 $\pm$ 5.24	75.5 $\pm$ 3.44
Total effluent load	9.35 $\pm$ 9.72	38.2 $\pm$ 19.7	6.55 $\pm$ 12.6	31.1 $\pm$ 13.3	3.45 $\pm$ 5.19	40.4 $\pm$ 16.6	12 $\pm$ 14.8	28.2 $\pm$ 25.6	8.29 $\pm$ 12.2	39.3 $\pm$ 29.3
Total load reduction	52.2	38.2	55.7	46.5	62.7	34.9	46.6	46.7	53.3	36.2
Load reduction (%)	84.8%	50.0%	89.5%	60.0%	94.8%	46.3%	79.6%	62.3%	86.5%	47.9%
Plant uptake	18.6	18.0	17.0	19.4	16.0	15.0	14.9	23.6	17.1	14.1
Plant uptake (%)	35.6%	47.3%	30.5%	41.7%	25.5%	43.1%	31.9%	50.6%	32.0%	39.1%
Other removal processes	33.6	20.1	38.7	27.1	46.7	19.8	31.8	23.1	36.2	22.0
Total phosphorus [mean $\pm$ SE (g·m ⁻³)]										
	4.5		5.5		6.5		7.2		8.5	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Material balance										
Total influent load	27.7 $\pm$ 1.43	32.1 $\pm$ 2.13	26.8 $\pm$ 1.35	32.2 $\pm$ 1.9	28.8 $\pm$ 2.5	31.7 $\pm$ 1.22	27.4 $\pm$ 2.28	35.2 $\pm$ 8.56	30.4 $\pm$ 5.39	32.3 $\pm$ 1.54
Total effluent load	3.96 $\pm$ 4.66	20.3 $\pm$ 7	5.77 $\pm$ 6.47	19 $\pm$ 5.53	7.87 $\pm$ 4.73	22.1 $\pm$ 4.71	10.8 $\pm$ 7.48	19.6 $\pm$ 6.56	10.4 $\pm$ 4.85	19.1 $\pm$ 7.29
Total load reduction	23.8	11.8	21.0	13.2	20.9	9.62	16.6	15.5	20.0	13.2
Load reduction (%)	85.7%	36.8%	78.4%	41.1%	72.7%	30.3%	60.4%	44.2%	65.9%	41.0%
Plant uptake	15.2	3.17	17.2	4.55	5.44	1.09	0.85	5.02	6.81	9.37
Plant uptake (%)	63.8%	26.8%	82.1%	34.4%	26.0%	11.3%	5.11%	32.3%	34.0%	70.8%
Other removal processes	8.60	8.65	3.76	8.69	15.5	8.53	15.7	10.5	13.2	3.87

^a1 g·m⁻³ = 0.0270 oz./yard³.

such as plants, biofilm, and algae, on nutrient composition and speciation was conducted, and the results were compared with results provided by the Visual MINTEQ model by averaging solution parameters from day 7 across the 6-week experiment in all planted EUs.

DATA ANALYSIS. When assessing changes in concentration on a weekly basis after each 7-d exposure to treatment loads, results were clustered by plant species and separated by pH level. Data are presented as loading rates to account for the amount of nutrients per unit surface area of the water covered by FTWs (grams per square meter). Initial nutrient loads varied weekly because adjustments to stock tanks resulted in fluctuations over the course of the experiment. Therefore, calculations of removal efficacy or percent of the nutrient removed resolved this variability.

A statistical model was developed that related nutrient removal levels to the treatments. An analysis of variance (ANOVA) was used to test the effect of the treatments on the nutrient removal means. When treatment was found to have an effect, then Student's *t* test was conducted to determine specific differences among the nutrient removal level means among the treatments. The ANOVA model included the EU and weekly variations as random effects and the pH treatments as a fixed effect. All statistical calculations were conducted using JMP (version 13; SAS Institute, Cary, NC). *P* < 0.05 was considered evidence of statistical significance.

Results and discussion

PLANT EFFECTS ON pH. Plant species impacted solution pH over the 7-d hydraulic retention time (*P* ≤ 0.001). Because trends were similar across 2016 and 2017, results from the two experiments were pooled for analysis and discussion (*P* > 0.05). The pH of solutions within the 'Rising Sun' japanese iris treatment was consistently lower on day 7 than those recorded for the other two plant species and the control for pH treatments 5.5, 6.5, 7.2, and 8.5 (*P* ≤ 0.05) (Fig. 2). Conversely, the pH of maidencane and bushy bluestem stabilized over the 7-d period, with the final pH close to the initial pH or neutral pH (Fig. 2). The pH of maidencane and bushy bluestem

Fig. 3. Effect of initial pH treatments of (A) 4.5, (B) 5.5, (C) 6.5, (D) 7.2, and (E) 8.5 on total ionic nitrogen (ammonium + nitrite + nitrate) removal efficacy averaged over two 6-week experiments as affected by three plant species (bushy bluestem, maidencane, and 'Rising Sun' japanese iris) compared with an unplanted control. Lines are averages ($n = 6$) for day 7 of a 7-d hydraulic retention time across two experiments. Results are means $\pm$ SE.

solutions differed from each other and from 'Rising Sun' japanese iris in the 5.5 and 6.5 pH treatments ($P \leq 0.05$). For all plant species evaluated in all pH treatments, except 4.5, the presence of plants decreased

the solution pH from that of the control.

Plants influenced how pH changed over the 7-d exposures. During this experiment, changes in the pH of the control were most likely attributed to the presence of algae within all control EUs. Visual observations of EUs established with plants showed less algae production over the experiment. In the presence of light, such as the conditions found in our greenhouse during sampling hours, algae absorb large amounts of carbon dioxide (CO_2), which is a weak acid (Jacob-Lopes et al., 2009). This decline in CO_2 as well as uptake of N compounds from solution may cause pH to increase during the day. Both maidencane and bushy bluestem prefer neutral (pH 7.0) growth sites (Newman et al., 2006a, 2006b). Irises (*Iris* sp.) prefer acidic soils for growth and nutrient uptake. The change in the pH of solutions planted with 'Rising Sun' japanese iris may be due to its capacity to acidify its root zone to work toward this ideal pH. The leaves and rhizomes of irises contain carboxylic acids, a plausible contributor to the acidification of the water surrounding the root system (Mikhailenko et al., 2018). It is plausible that through one of the aforementioned processes, 'Rising Sun' japanese iris may be able to reduce the pH around it. Additional studies are needed to document the exact process by which irises induce root zone acidification and the effects of hydraulic retention time and flow rate on pH change.

pH EFFECT ON PLANT-AIDED NUTRIENT REMEDIATION. Total ionic N ($\text{TN} = \text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$) and P material balance and load reduction differed from 2016 to 2017 for all plant species ($P \leq 0.01$). Therefore, results related to load are presented by year. Material balance calculations were performed and reductions in cumulative load over the two 6-week experiments determined the incorporated plant contribution to nutrient removal (Tables 2–4). Trends in the percent removal efficacy of N and P were similar between 2016 and 2017 ($P > 0.05$); therefore, differences in nutrient concentrations from day 0 (influent concentration) to day 7 (final effluent concentration) were calculated and reported as the percent removal averaged

Fig. 4. Effect of initial pH treatments of (A) 4.5, (B) 5.5, (C) 6.5, (D) 7.2, and (E) 8.5 on phosphate removal efficacy averaged over two 6-week experiments as affected by three plant species (bushy bluestem, maidencane, and 'Rising Sun' japanese iris) compared with an unplanted control. Lines are an average ($n = 6$) for day 7 of a 7-d hydraulic retention time across two experiments. Results are means $\pm$ SE.

across the 6 weeks of both experiments (Figs. 3 and 4), accounting for the concentration of nutrients within the water column itself. To assess the

plant contribution to nutrient removal, cumulative plant uptakes of nutrients over the 6-week experiments were calculated as the final

harvest plant tissue nutrient concentration minus the plant tissue nutrient concentration at experiment initiation, when the FTW was planted, according to the dry weight of the plant (Figs. 5 and 6).

Bushy bluestem reduced the TN load from that of the influent of all pH treatments ($P \leq 0.05$) (Table 2). Cumulative load reduction was lowest in pH 8.5 treatments for both years (mean $\pm$ SE; $55.6\% \pm 15.2\%$ and $22.7\% \pm 8.34\%$) and greatest in pH 4.5 treatments ($82.1\% \pm 9.8\%$ and $48.7\% \pm 5.1\%$), with similar removal within the other pH treatments ($P > 0.05$) (Table 2). In the lower pH treatments (4.5 and 5.5), bushy bluestem consistently removed more than 40% of the N available in solution over the 6-week trials (Fig. 3). However, as treatment pH increased above 6.5, TN removal efficacy declined over 6 weeks, averaging only $20\% \pm 9.3\%$ and $39\% \pm 4.2\%$ for the 7.2 and 8.5 pH treatments, respectively. This decline could be attributed to a decrease in the growth and vigor of the bushy bluestem within the 7.2 and 8.5 pH treatments over the 6-week period and the increasing presence of algae competing for nutrients. The loss of TN removal efficacy in higher pH treatments (7.2 and 8.5) was further explained by the relatively low capacity of bushy bluestem tissues to accumulate TN in these higher pH treatments. In the pH 7.2 treatments, TN accumulation within plant tissue was only $10.6 \text{ g} \cdot \text{m}^{-2}$ in 2016; in 2017, it was $7.80 \text{ g} \cdot \text{m}^{-2}$. In pH 8.5 treatments, TN accumulation within plant tissue was only $12.8 \text{ g} \cdot \text{m}^{-2}$ in 2016; in 2017, it was only $4.38 \text{ g} \cdot \text{m}^{-2}$ (Fig. 5). The pH did not affect aqueous P load reduction ($P > 0.05$) (Fig. 4), but plant P uptake was variable across pH levels ($P \leq 0.01$ for both years), suggesting that other removal processes were driving P removal and possibly algal removal or precipitation of P (de-Bashan and Bashan, 2004). The greatest plant uptake of P occurred in the pH 4.5 treatments in 2016 ($2.24 \text{ g} \cdot \text{m}^{-2}$; $P = 0.004$) and in the pH 6.5 treatments in 2017 ($1.02 \text{ g} \cdot \text{m}^{-2}$; $P = 0.013$) (Fig. 6).

Maidencane performance across pH treatments was the most variable of the three species of plants screened, with TN removal varying from 4.58% to 91.6% according to pH treatment and year ($P < 0.01$) (Table 3). For

Fig. 5. Plant uptake of total nitrogen by three species (A) bushy bluestem, (B) maidencane, and (C) 'Rising Sun' japanese iris at the conclusion of a 6-week exposure to nutrients in floating treatment wetlands at five pH levels (4.5, 5.5, 6.5, 7.2, 8.5) for 2 years (2016 and 2017). Results are means $\pm$ SE; $1 \text{ g}\cdot\text{m}^{-2} = 0.0033 \text{ oz}/\text{ft}^2$.

both years, removal of both TN and P ($P \leq 0.001$ for years and nutrients), except for PO_4^{3-} in 2017 ($P \leq 0.05$), was influenced by the pH treatment. In 2016, maidencane had the greatest

TN load reduction in pH 4.5 and 5.5 treatments (56.4 and $55.2 \text{ g}\cdot\text{m}^{-2}$) ($P > 0.05$). These results were partly due to the consistent and increasing removal efficiency of TN aided by

maidencane over the 6-week period for pH 4.5 (average efficacy, 75%) and 5.5 treatments (average efficacy, 66%) (Fig. 3). In pH treatments 6.5, 7.2, and 8.5, removal of TN occurred until week 5 for 6.5 and 8.5 and until week 3 for pH 7.2. Despite its low performance at higher pH treatments, maidencane was the top performer in 2016 regarding both TN removal efficacy and N accumulation within plant tissues compared with the other two species, with plant uptake of $24.1 \text{ g}\cdot\text{m}^{-2}$ at pH 4.5 ($P < 0.05$) (Fig. 5). In both 2016 and 2017, P remediation by maidencane was maximized at pH 4.5 and 7.2 ($P < 0.05$), whereas the remaining pH treatments reduced P to a similar degree ($P > 0.05$) (Table 3). Although TN removal efficacy decreased over the 6-week period for higher pH treatments, overall removal efficacy for PO_4^{3-} was comparatively stable over time for all pH treatments (Fig. 5).

Unlike the two other species used in this experiment, pH did not influence the TN load reduction of 'Rising Sun' japanese iris in either 2016 or 2017, or the P load reduction in 2017 ($P > 0.05$ for all) (Table 4). These are important results because they indicate that the nutrient remediation performance of 'Rising Sun' japanese iris in FTWs may be less influenced by the pH of the system, making it a suitable plant recommendation for use in FTWs, regardless of the pH of the water in the system in which it is deployed. Furthermore, performance of 'Rising Sun' japanese iris was consistently higher than that of the two other species ($P \leq 0.01$) regarding both TN and P load reduction and removal efficacy, with the exception of pH 4.5, where the three types of plants performed similarly ($P > 0.05$) (Table 4, Figs. 3 and 4).

In 2017, across all pH treatments, except 7.2, there was a decrease in plant uptake of both TN and P for maidencane and bushy bluestem in comparison with their 2016 removal rates ($P \leq 0.05$). However, 'Rising Sun' japanese iris continued to demonstrate plant uptake rates similar to or exceeding those of the 2016 experiment ($P \leq 0.05$ for TN 7.2 and P 4.5, 5.5, 7.2, and 8.5; $P > 0.05$ for all others) (Figs. 5 and 6). Several studies (Keizer-Vlek et al., 2014; Vymazal, 2007; Yousefi and Mohseni-Bandpei, 2010)

Fig. 6. Plant uptake of phosphorus by three species (A) bushy bluestem, (B) maidencane, and (C) 'Rising Sun' japanese iris at the conclusion of 6-week exposure to nutrients in floating treatment wetlands at five pH levels (4.5, 5.5, 6.5, 7.2, 8.5) for 2 years (2016 and 2017). Results are means $\pm$ SE; $1 \text{ g}\cdot\text{m}^{-2} = 0.0033 \text{ oz}/\text{ft}^2$.

evaluated yellow flag iris (*Iris pseudocorus*) and found that it often outperformed other species used for nutrient remediation applications in wetlands. These findings concur and

suggest that the performance potential may extend to other irises.

VISUAL MINTEQ SIMULATIONS. Visual MINTEQ models were initially performed with values (Table

1) obtained from near-neutral (7.2) municipal water treated with fertilizer to attain concentrations of $12 \text{ mg}\cdot\text{L}^{-1}$ N. This baseline was then modeled at different pH levels (4.5, 5.5, 6.5, and 8.5), including the addition of any chemicals used to adjust the solution pH, and compared with mesocosm observations. The pH influenced the Visual MINTEQ-predicted concentrations of orthophosphate (H_2PO_4^- and HPO_4^{2-}) within the simulated runoff, but the experimental concentration of PO_4^{3-} in the EUs was not influenced by the solution pH ($P \geq 0.05$) (Fig. 7). These modeled and experimental PO_4^{3-} results according to pH were contrary to that found by Cerozi and Fitzsimmons (2016), who reported that increasing pH reduced the availability of PO_4^{3-} within the system. Although the Visual MINTEQ simulation overpredicted or underpredicted the level of PO_4^{3-} within the experiment, the experimental concentrations of PO_4^{3-} consistently demonstrated decreasing PO_4^{3-} concentrations as pH increased (Cerozi and Fitzsimmons 2016). Our results were inverse, with modeled PO_4^{3-} concentrations increasing with pH. This would suggest that, in our model, Fe controlled P dissolution within the system to a greater effect than Ca. This could be attributed to the high level of Fe ($0.3 \text{ mg}\cdot\text{L}^{-1}$ Fe) within the modeled water system compared with calcium ($8.44 \text{ mg}\cdot\text{L}^{-1}$ Ca). However, experimental concentrations remained steady across pH treatments. Evaluation of the percent distribution of PO_4^{3-} species from the model indicated that at low pH levels, a large percent of PO_4^{3-} existed in a soluble form [aluminum phosphate (AlHPO_4^+)]. AlHPO_4^+ was excluded from the presented PO_4^{3-} simulated values because it was not considered available for plant uptake; therefore, inclusion would have increased PO_4^{3-} concentrations at pH levels of 4.5 and 5.5. Although the modeled systems are designed to reach equilibrium, the natural systems are not typically at equilibrium, which is a possible reason for some of the differences in predicted and experimental concentrations (Butcher, 1992). The reduction of orthophosphate following the introduction of

Fig. 7. Modeled, experimental (day 0), and biological impact (day 7, floating treatment wetlands) on concentrations of (A) phosphate, (B) nitrate, (C) nitrite, and (D) ammonium at different pH levels in runoff solutions. Results are means $\pm$ SE separated by Student's *t* in which levels not connected by the same letter significantly differ at $P \leq 0.05$ for experimental and biological values (*n* is variable across factors) with model results of *n* = 1; therefore, no SE was reported. Modeled data were generated using Visual MINTEQ 3.0 (Gustafsson, 2012); 1 mg·L⁻¹ = 1 ppm.

plant systems was similar across pH levels ($P \geq 0.05$) (Fig. 7).

The three N species that were measured, NH_4^+ , NO_2^- , and NO_3^- , were consistent across simulated pH levels, with a slight decrease at pH 8.5 in NH_4^+ (Fig. 7). The impact of FTWs further decreased the presence of NO_3^- in solution across pH treatment, with the greatest decrease occurring at pH 4.5 and the least decrease occurring at 6.5 ($P \leq 0.05$). Both experimental NO_2^- and NH_4^+ were affected by pH ($P \leq 0.05$). Predicted NO_2^- concentrations were 4.6×10^{-12} mg·L⁻¹ across pH levels, which was below the detection level (0.02 mg·L⁻¹) for the analysis. Therefore, pH 4.5 and 5.5 experimental values were considered nondetectable, but they may be comparable to the modeled level (Fig. 7C). At pH ≥ 6.5 , NO_2^- experimental concentrations

increased in comparison with pH ≤ 5.5 . This increased presence was also found for the biological impact (planted mesocosms) on NO_2^- concentrations ($P \leq 0.05$) (Fig. 7). Conversely, experimental and biological concentrations of NH_4^+ were significantly lower at high pH compared with lower pH levels ($P \leq 0.05$ for both) (Fig. 7).

Increases in NO_2^- could be attributed to nitrification, or the oxidation of NH_4^+ to NO_2^- , a process that is largely dependent on dissolved oxygen levels (Garcia Chance and White, 2018). Differences in dissolved oxygen were not found between pH levels ($P \geq 0.05$). However, the bacteria responsible for the conversion of NH_4^+ to NO_2^- (*Nitrosomonas*) is most active at pH > 7 , with almost complete cessation of NH_4^+ oxidation to NO_2^- at pH < 6 (Fumasoli et al., 2015). This is

further confirmed by the decreasing levels of NH_4^+ at increasing pH levels ($P \leq 0.05$) (Fig. 7). The presence of plant systems within the EUs would further promote biofilm formation and the growth of bacteria communities, which support the experimental effects on concentrations (Fig. 7). Both NO_3^- and NH_4^+ are readily taken-up by plants; therefore, a large amount of the decrease from the experimental to biological impact data would be attributed to the presence of plants (Fig. 7). As indicated through this research, the amount of N removed by plant presence is highly dependent on the plant taxa used within the system.

Conclusion

Results from model simulations poorly predicted the nutrient availability in effluent, likely because

plant-mediated and bacteria-mediated contributions were not included in the model. The pH of water in which plant-based treatment systems, such as FTWs, are deployed is important. The pH conditions of the water must be considered when designing these treatment systems because plant selection affects system performance. The pH influenced both N and P removal efficacy in FTW systems for two of the three species of plants studied, maidencane and bushy bluestem. Higher pH decreased the amount of N and P removed from water over the experimental period, but plants were still capable of absorbing nutrients into their tissues across all pH levels. Both maidencane and bushy bluestem performed best at low pH, specifically 4.5, with maidencane removing as much as 90% of the total N load from the system. Therefore, for FTWs deployed in water bodies where pH is consistently low, all three species of plants are good selections. 'Rising Sun' Japanese iris maintained similar N and P removal efficacies and load reductions across all experimental pH levels. The high N and P load reduction facilitated by 'Rising Sun' Japanese iris showed its adaptability, resilience, and potential for use in highly variable or extreme runoff conditions.

Overall, nutrient remediation performance for all species of plants evaluated was ideal at the pH levels of 4.5–5.5, which is lower than the pH range of 5.5–7.2 pH that is typically recommended for hydroponics systems (Cerozi and Fitzsimmons, 2016). 'Rising Sun' Japanese iris consistently lowered the pH of the influent for all pH treatments, indicating the potential for the plant-induced pH change to enhance consistency of nutrient remediation. All planted systems resulted in more stable pH conditions compared with the pH of the open control systems. The stability could be due to suppression of algal blooms and diurnal pH fluctuations caused by their metabolic processes (photosynthesis and respiration).

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