Alkalinity of Irrigation Return Water Influences Nutrient Removal Efficacy of Floating Treatment Wetland Systems[1]

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Abstract

Water quality concerns often prevent reuse of captured irrigation return water for irrigation of specialty crops. Prior research indicated alkalinity of specialty crop operation irrigation varies from 0 to >500 $mg \cdot L^{-1}$ (>0.06 $oz \cdot gal^{-1}$) $CaCO_3$ across the United States. Floating treatment wetlands (FTWs) are an option for remediation of nutrients in irrigation return water, but effects of variable alkalinity on nutrient removal efficiency of FTWs are unknown. An experimental FTW system was developed to quantify the effect of alkalinity on the growth and nutrient uptake capacity of three plant species. 'Rising Sun' Japanese iris (*Iris ensata* 'Rising Sun' Thunb.), upright sedge (*Carex stricta* Lam.);, and switchgrass (*Panicum virgatum* L.). were grown for 6 weeks at one of five alkalinity treatment levels, representing the alkalinity range of nursery and greenhouse irrigation runoff: 0, 100, 200, 300, and 400 $mg \cdot L^{-1}$ $CaCO_3$ (0, 0.01, 0.02, 0.04, 0.05 $oz \cdot gal^{-1}$ $CaCO_3$). Overall, Japanese iris demonstrated consistent remediation across each alkalinity treatment for both nutrient load reduction and plant accumulation. Species of iris warrant greater consideration and use in bioremediation systems. Both upright sedge and switchgrass could be used in systems with appropriate alkalinity levels. Future work should consider assessing novel plants at different points within their growth cycle, extended exposure durations, and decreased hydraulic retention time.

Index words: Aquatic plant, nitrogen, phosphorus, sodium bicarbonate, nitrogen speciation.

Species Used in this study: 'Rising Sun' Japanese iris (*Iris ensata* 'Rising Sun' Thunb.); upright sedge (tussock sedge) (*Carex stricta* Lam.); switchgrass (*Panicum virgatum* L.).

Significance to the Horticulture Industry

The quality of water obtained from municipal, municipal reclaimed, surface- or ground- water sources may vary seasonally and with changes in upstream practice (e.g., herbicide residues from upstream application could enter irrigation water). Unmanaged changes in water quality could result in crop losses due to presence of plant pathogens, excess salts (foliage burns), and pesticide residues. The capacity to (1) remediate irrigation return water and water added from other sources, (2) store the water onsite, and (3) reuse water for plant production could mean the difference between business success or failure, especially during drought events.

Floating treatment wetlands hold promise for remediation of acidic or alkaline water without the need for expensive injection systems (e.g., acid or base). Growers need better information relating how water chemistry (pH and EC) influences plant nutrient uptake (both in production systems and with regard to treatment efficacy), as conventions regarding nutrient fate and soil chemistry may not translate directly to aqueous systems. Floating treatment wetland systems can also be used to cleanse production return water prior to release offsite, limiting potential for negative environmental consequences, aiding with compliance to current and future regulations related to capture and treatment of production return water.

Introduction

As the specialty crops industry works to reduce costs while increasing profitability, irrigation water management and the capture and reuse of irrigation return flows (i.e., the water that flows off production areas) on site is a critical point of consideration. Treating irrigation return flow water to increase viability for irrigation reuse has resulted in the implementation of many treatment technologies by specialty crop growers (Majsztrik et al. 2017). Floating treatment wetland systems (FTWs) are a type of treatment system that effectively reduce nitrogen (N) and phosphorus (P) loads in surface water; reduced nutrient loads in turn limit aquatic weed growth (Jones et al. 2017). FTWs consist of a buoyant mat suspended on the surface of the water body, holding macrophytes in place with the shoots held above water and roots suspended within the water column. Direct exposure of the roots to the water column facilitates direct nutrient absorption by both wetland plants and root-associated microbial communities (Garcia Chance and White 2018, White and Cousins 2013). Exposure of FTWs to nursery and greenhouse irrigation return flows is potentially different from other FTW applications because of the large range of nutrient concentrations and the

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variability of water characteristics, such as pH and alkalinity.

Irrigation return water from nurseries and greenhouses has nutrient concentrations ranging from 0.1 to 387 mg·L^{-1} NO_3^-N, 0.9 to 47 mg·L^{-1} ammoniacal-nitrogen (NH_4^+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). Argo et al. (1997) conducted a geographical analysis of irrigation water applied to greenhouse operations across the United States and Canada and found the alkalinity of water applied as irrigation ranged from 0 to 1,120 mg·L^{-1} $CaCO_3$. The mean alkalinity of all water samples was 160 mg·L^{-1} $CaCO_3$ with a median of 200 mg·L^{-1} $CaCO_3$ in samples from Illinois and Michigan; overall, the alkalinity measured for the samples was uniformly distributed over the full range. The alkalinity of applied irrigation water does not directly translate to the alkalinity of greenhouse or nursery irrigation return water. In a study conducted by Copes et al. (2017) assessing nursery and greenhouse containment basins receiving irrigation return water in five southeastern states, alkalinity varied from 0 to 140 mg·L^{-1} $CaCO_3$. It is important to note that changes in alkalinity from irrigation application to return flow largely depend upon the site's geography and geology, dilution from rainfall, and impact of algae or other biological matter within irrigation lines or containment basins (Chen et al. 2003).

Alkalinity is a measure of water's buffering capacity and when alkalinity is high it can cause an increase in pH by neutralizing H^+ (Kuehny and Morales 1998). One of the most obvious responses of plants to high alkalinity is stunted growth and the induction of interveinal chlorosis in the plant's youngest leaves (Lucena 2000). Alkalinity-induced leaf chlorosis has been attributed to an iron (Fe) deficiency due to decreased Fe uptake and/or diminished Fe availability (Bertoni et al. 1992). Fe is required for the synthesis of the heme structure, the essential part of chlorophyll (Borlotti et al. 2012). If Fe in the plant is not available or inadequate, the synthesis of chlorophyll is impaired. Alkalinity can reduce the solubility of Fe. At high pH values, Fe forms hydroxides or other insoluble compounds (Valdez-Aguilar and Reed 2007). High alkalinity in water can be harmful to plant growth and development. Water with little to no alkalinity has no buffering capacity, and thus can experience sudden changes in pH due to environmental or biotic constraints, which may also impact nutrient availability (Whipker et al. 1996). The maximum alkalinity that a plant can tolerate depends on plant species, the age of the plant, type of growing medium used, length of the crop production cycle, growing medium volume and buffering capacity, and irrigation management practices (Valdez-Aguilar and Reed 2007). In general, acceptable levels of alkalinity in irrigation water varies between 0 and 160 mg·L^{-1} HCO_3^-, with 30 to 60 mg·L^{-1} HCO_3^- considered ideal for most plants (Mattson 1995, Roosta 2011, Valdez-Aguilar and Reed 2007).

A previous study by Garcia Chance et al. (2019) assessed how pH influenced the nutrient removal efficacy of FTWs and determined some species of plants may tolerate exposure to a greater range of pH while still contributing to efficient removal of nutrients from water. While water pH and alkalinity can be related, a survey of 192 Ohio groundwater samples showed no correlation between the two variables (Altland 2018). Water with high alkalinity typically has a high pH (7 or higher), but high water pH does not necessarily result in high alkalinity (Mattson 1995). The majority of studies assessing the role of alkalinity in nutrient availability or plant growth and development have been conducted in soilless substrates, with very few in hydroponic evaluations. Anderson et al. (2017) looked at two alkalinity levels, 20 and 40 mg·L^{-1} $CaCO_3$, in pH 7 solutions and found no difference in nutrient availability or plant growth for 'Flandria' lettuce (*Lactuca sativa* Linn. cv. Flandria) while Roosta (2014) found increased production of strawberry (*Fragaria ×ananasa* Weston*)* with an increase in ammonium : nitrate ratios for 500 to 900 mg·L^{-1} $NaHCO_3$. The conditions within the few experimental hydroponic systems do not accurately mimic those to which FTWs may be exposed, especially the potential for a wide range of alkalinity levels. Hydroponic studies also typically look at factors outside those of interest to remediation applications – mainly, the efficiency of nutrient removal from solution.

The goal of this study was to determine if alkalinity impacted the efficiency of N and P remediation aided by FTWs established with three species of plants. This goal was assessed through two objectives. The first objective was to quantify the plant tissue nutrient content, or plant uptake contribution, when exposed to five levels of alkalinity. The second objective was to quantify the nutrients remaining within the water source, or final nutrient remediation efficiency from simulated irrigation return water, at five alkalinity levels. By evaluating these objectives, a fuller understanding of alkalinity impact on nutrient allocation can be developed.

Materials and Methods

Experimental setup. The experiment was repeated using two, 6-week studies (June 22 – August 3, 2016 and June 16 -July 28, 2017). An experimental system was assembled at the Water Treatment Technology Laboratory at the South Carolina Water Resources Center at Clemson University (Pendleton, South Carolina, USA, 34.640N, -82.773W), comprised of fifty, 37.9 L (10 gal) plastic tubs (United Solutions Rough and Rugged, Leominster, MA). Each tub 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, 420-L tanks (Poly-Mart Vertical Water Storage) were plumbed with PVC lines and served as holding tanks for each alkalinity treatment. The alkalinity of the water in each holding tank was adjusted to the appropriate treatment level, as selected based upon nursery effluent ranges: 0 (baseline), 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$, and thoroughly mixed prior to filling individual tubs. A water hose connected to a pump was installed at the bottom outlet of each holding tank, which permitted filling of the tubs.

One-centimeter-thick floating mats, supplied by Beemats (New Smyrna Beach, FL), were cut to three 10 cm (4 in) by

Fig. 1. Experimental setup for floating treatment wetland experiments evaluating the influence of alkalinity (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$) and plant species ('Rising Sun' Japanese iris, switchgrass, and upright sedge) remediation of nutrients from simulated nursery runoff. Fifty experimental units were randomly assigned a treatment combination or as an alkalinity control with no plants present. The five holding tanks permitted ease of mixing and consistent alkalinity exposures for treatments each time the experimental units were refilled.

10 cm squares with 7.5 cm (3 in) pre-cut holes located in the center of the cut mat for each tub. The holes allowed insertion of specially designed aerator cups in which plants were placed. Three species of plants were used in this study, upright sedge, 'Rising Sun' Japanese iris, and switchgrass. Japanese iris were supplied as rhizomes (Supplier A [southeast] in 2016 and 2017) and upright sedge and switchgrass were sourced as bare root liners (Supplier B [northeast] in 2016 and Supplier C [southeast] in 2017).

Treatments were assigned to tubs using a completely randomized design. For each tub, three plants of one species were placed into aerator cups with 15 tubs allocated to each species (Fig. 1), and three tubs were assigned to each plant species per alkalinity treatment level. One additional control tub with no plants was filled with each alkalinity solution for a total of 50 tubs. Plants were grown for six weeks to determine nutrient uptake efficacy prior to harvest.

Simulation of irrigation return water containing nutrients. To simulate nursery return water, municipal water was spiked with water soluble fertilizer to attain concentrations of 12 mg·L^{-1} N by adding and dissolving 72.6 g of a water-soluble fertilizer (20N-2P-20K Nitrate Special Soluble Fertilizer, Southern Agricultural Insecticides, Inc., Hendersonville, NC) within each 420-L water storage tank. The alkalinity of the municipal water was 16 mg·L^{-1} $CaCO_3$ and was used as the baseline 0 mg·L^{-1} $CaCO_3$ treatment. The alkalinity treatments were attained by dissolving sodium bicarbonate ($NaHCO_3$) into each storage tank, to increase the alkalinity to target levels of 100 (63.4 g $NaHCO_3$), 200 (126.8 g $NaHCO_3$), 300 (221.9 g $NaHCO_3$), and 400 mg·L^{-1} $CaCO_3$ (317.0 g $NaHCO_3$). Nutrient and alkalinity solutions were newly created every 7 days. Each tub was filled from its designated holding tank on Day 0 and then drained on Day 7 (static renewal) to simulate a 7-d hydraulic retention time (HRT) over the six-week experiments.

Alkalinity adjustments were made using sodium bicarbonate ($NaHCO_3$), a low-cost material that can be purchased in bulk, a necessity given the mass needed to adjust alkalinity levels to the points needed over the experiment. To avoid confounding of nutrient availability, ammonium bicarbonate (NH_4HCO_3) and potassium bicarbonate ($KHCO_3$), two macronutrients needed for plant growth, were not used. Plant nutrient availability studies commonly use $NaHCO_3$ to adjust alkalinity adjusted levels because it is easy to procure and apply (Roosta et al. 2016, Valdez-Aguilar and Reed 2010, Valdez-Aguilar and Reed 2007). A by-product of the use of $NaHCO_3$ is potential for the dissolution of Na within the solution, elevating Na levels that may either accumulate within plant tissues or negatively impact cation balance in solutions, disrupting metabolic processes, such as stomatal regulation, that require low Na^+ and high K^+ to function (Tank and Saraf 2010). Therefore, effects of Na upon plant growth and nutrient availability were also considered.

Water sampling and analysis. Water samples were collected from the storage tanks each week on Day 0, after the fertilizer and $NaHCO_3$ amendments were completely dissolved and mixed for baseline water analysis. For each tub, water samples were collected on Day 7 for six weeks. Additional water samples were collected on Day 3 and 5 every two weeks (Week 2, 4, and 6). 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, while IC water samples were filtered using a 0.22 µm Luer lock filter (Whatman GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) and then placed in a -25 C freezer. Trace elements including P, K, Ca, Mg, Zn, Cu, Mn, Mo, Ni, Fe, S, Na, B, and Al were analyzed via ICP-OES (iCAP 6500, Thermo Scientific, Waltham, MA). Anions, including ammonium, nitrate, nitrite, phosphate, and sulfate, were measured using a Dionex AS10 ion chromatograph with AS50 auto-sampler (Dionex Corp., Sunnyvale, CA, USA). All analyses were conducted according to US EPA protocol methods 6010B and 9056A and calibration standards were instituted for quality assurance and control (USEPA 1997, USEPA 2007). Environmental parameters, including pH and temperature (C), were measured in a consistent manner using a calibrated, handheld multi-meter (YSI, Yellow Springs, OH) on Day 0, 3, 5, and 7 each week, for six weeks. Alkalinity was measured by titrating to a pH endpoint of 4.5 using 0.02 N (0.01 M) sulfuric acid to an accuracy of ±4 mg·L^{-1} as $CaCO_3$. All samples were collected in the morning between 0700 and 0900, at a depth of 15 cm in each tub.

Plant sampling and analysis. The roots (below-mat biomass) and shoots (above-mat biomass) of three plants per species were harvested prior to each experiment start date to provide baseline nutrient status for each plant species (Table 1). At the end of the 6-week period, one plant from each planted tub (n=45) was harvested to

Table 1. Initial (pre-experiment) and final average root length (cm), shoot length (cm), and dry mass (g) for 'Rising Sun' Japanese iris, switchgrass, and upright sedge across 5 alkalinity treatments (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$ using sodium bicarbonate) for two years (2016 and 2017) after 6-week exposure to nutrients in floating treatment wetlands. Values presented are the means (standard error) of the mean.

	2016			2017		
Avg. Root Length (cm)	**Upright sedge**	**Japanese iris**	**Switchgrass**	**Upright sedge**	**Japanese iris**	**Switchgrass**
Alkalinity Level (mg·L^{-1})						
Initial	18.3 ± 2.93	8.50 ± 3.77	16.5 ± 1.80	30.8 ± 11.1	18.3 ± 6.05	11.2 ± 1.04
0	16.5 ± 9.10	15.2 ± 2.47	16.2 ± 8.69	24.0 ± 1.73	32.7 ± 11.0	8.33 ± 1.15
100	21.2 ± 12.3	20.0 ± 11.5	17.8 ± 2.25	14.7 ± 5.51	30.0 ± 9.54	12.0 ± 4.58
200	20.7 ± 2.52	11.8 ± 1.89	22.3 ± 16.4	12.7 ± 1.15	35.0 ± 1.00	11.7 ± 2.08
300	18.2 ± 5.20	13.5 ± 1.80	12.7 ± 0.76	14.0 ± 5.29	33.0 ± 9.54	11.3 ± 5.51
400	12.2 ± 2.36	15.3 ± 6.11	17.3 ± 6.66	12.7 ± 5.03	29.7 ± 8.33	11.7 ± 4.04
Avg. Shoot Length (cm)	**Upright sedge**	**Japanese iris**	**Switchgrass**	**Upright sedge**	**Japanese iris**	**Switchgrass**
Alkalinity Level (mg·L^{-1})						
Initial	36.7 ± 7.22	8.33 ± 3.79	34.0 ± 1.80	15.8 ± 3.62	68.8 ± 5.80	33.3 ± 4.07
0	64.7 ± 20.8	62.3 ± 16.5	69.7 ± 1.53	57.0 ± 24.1	71.7 ± 6.51	39.7 ± 7.51
100	54.0 ± 29.6	71.3 ± 16.7	76.0 ± 4.36	49.0 ± 6.08	61.0 ± 14.9	39.0 ± 21.2
200	47.3 ± 10.8	72.0 ± 7.94	65.0 ± 17.1	55.0 ± 3.00	66.3 ± 3.06	45.0 ± 3.61
300	77.3 ± 22.7	48.5 ± 17.4	71.7 ± 9.07	44.3 ± 7.23	71.0 ± 3.61	38.3 ± 6.35
400	78.0 ± 17.7	61.0 ± 22.9	69.3 ± 16.0	54.0 ± 9.54	61.3 ± 0.58	33.7 ± 3.21
Avg. Dry Weight Whole Plant (g)	**Upright sedge**	**Japanese iris**	**Switchgrass**	**Upright sedge**	**Japanese iris**	**Switchgrass**
Alkalinity Level (mg·L^{-1})						
Initial	2.40 ± 0.75	3.02 ± 3.35	0.73 ± 0.28	0.67 ± 0.44	3.55 ± 3.70	1.61 ± 0.45
0	11.5 ± 8.70	9.31 ± 9.81	9.17 ± 3.85	2.16 ± 2.15	7.28 ± 3.11	1.57 ± 0.57
100	7.40 ± 6.08	12.4 ± 12.7	13.7 ± 4.89	2.55 ± 1.28	5.19 ± 4.99	1.74 ± 0.88
200	4.00 ± 3.54	17.6 ± 8.53	10.6 ± 7.74	2.70 ± 1.08	8.51 ± 2.94	1.74 ± 0.50
300	10.9 ± 9.16	5.11 ± 4.33	13.7 ± 9.55	1.72 ± 0.58	9.05 ± 2.43	1.76 ± 0.78
400	12.5 ± 8.95	8.51 ± 11.4	9.74 ± 6.62	2.42 ± 1.46	8.07 ± 4.31	2.09 ± 0.74

quantify the change in tissue nutrient composition. The harvest process included measurement of the shoot height (cm), root length (cm), and width (cm) in two directions, followed by separation of the roots and shoots. Roots and shoots were weighed (g fresh weight), dried at 80 C, weighed (g dry weight), and ground in a Wiley mill (Swedesboro, NJ) to pass through a 40-mesh screen (0.425-mm). Carbon and nitrogen (total) in plant tissue were determined by flash-combustion and GC separation [NC analyzer (CN soil flash EA1112, CE Elantech Inc., Lakewod, N.J.)]. Operational parameters were 900 and 850 C for the primary and secondary column furnace. The column oven was set at 50 C. Sample incendiary gas was oxygen at 250 mL per minute at sample ignition with the carrier gas helium at 140 mL per minute. The instrument was standardized on BBOT (6-7% N and 65-80% C) with tomato leaf tissue (dried and ground) acquired from the National Institute of Standards and Technology (guaranteed elemental analysis) serving as the quality control check. Phosphorus, K, Ca, Mg, Zn, Cu, Mn Fe, S, Na, B, and Al concentrations in plant tissues were determined by ICP-OES with calibration standards rerun at the midpoint and end of each analytical run.

Data Analysis. When assessing changes in concentration on a weekly basis after each 7-day exposure to treatment loads, results were clustered by plant species and separated by alkalinity level. Data are presented as loading rates to account for the mass of nutrients per unit surface area of the water covered by FTWs (g·m^{-2}). Initial nutrient loads varied by week, as adjustments were made to achieve appropriate nutrient and alkalinity solution concentrations within the stock tanks over the course of the experiment. Therefore, calculations of removal efficacy or percent of the nutrient removed were used to resolve this variability.

A statistical model was developed that related nutrient removal levels to the treatments. Analysis of Variance (ANOVA) was used to test the effect of the treatments on nutrient removal means. When a treatment had an effect, then a Student's t-test was conducted to determine specific differences among the nutrient removal level means among the treatments. The ANOVA model included the tub and week to week variation as random effects and the alkalinity treatments as a fixed effect. All statistical calculations were conducted using JMP v13 (SAS Institute Inc., Cary, NC) and p-values < 0.05 were considered evidence of statistical significance.

Results and Discussion

Alkalinity and pH of the system. Neither plant species nor time (day or week) impacted solution alkalinity over the experiment duration ($p \geq 0.05$). Of the plant species trialed, none changed the alkalinity of the water solution (data not shown), indicating management of alkalinity may not be possible with FTWs. However, as anticipated, alkalinity treatment impacted pH ($p \leq 0.001$). The pH of treatment 0 mg·L^{-1} $CaCO_3$ averaged 7.14 ± 0.33, and pH gradually increased with each alkalinity treatment to reach a final average pH of 8.26 ± 0.10 at 400 mg·L^{-1} $CaCO_3$ (Fig. 2). Over the 7-day HRT, Japanese iris changed solution pH in 2016 and 2017 ($p \leq 0.001$). In 2016, Japanese iris increased solution pH on average by 0.68 ±

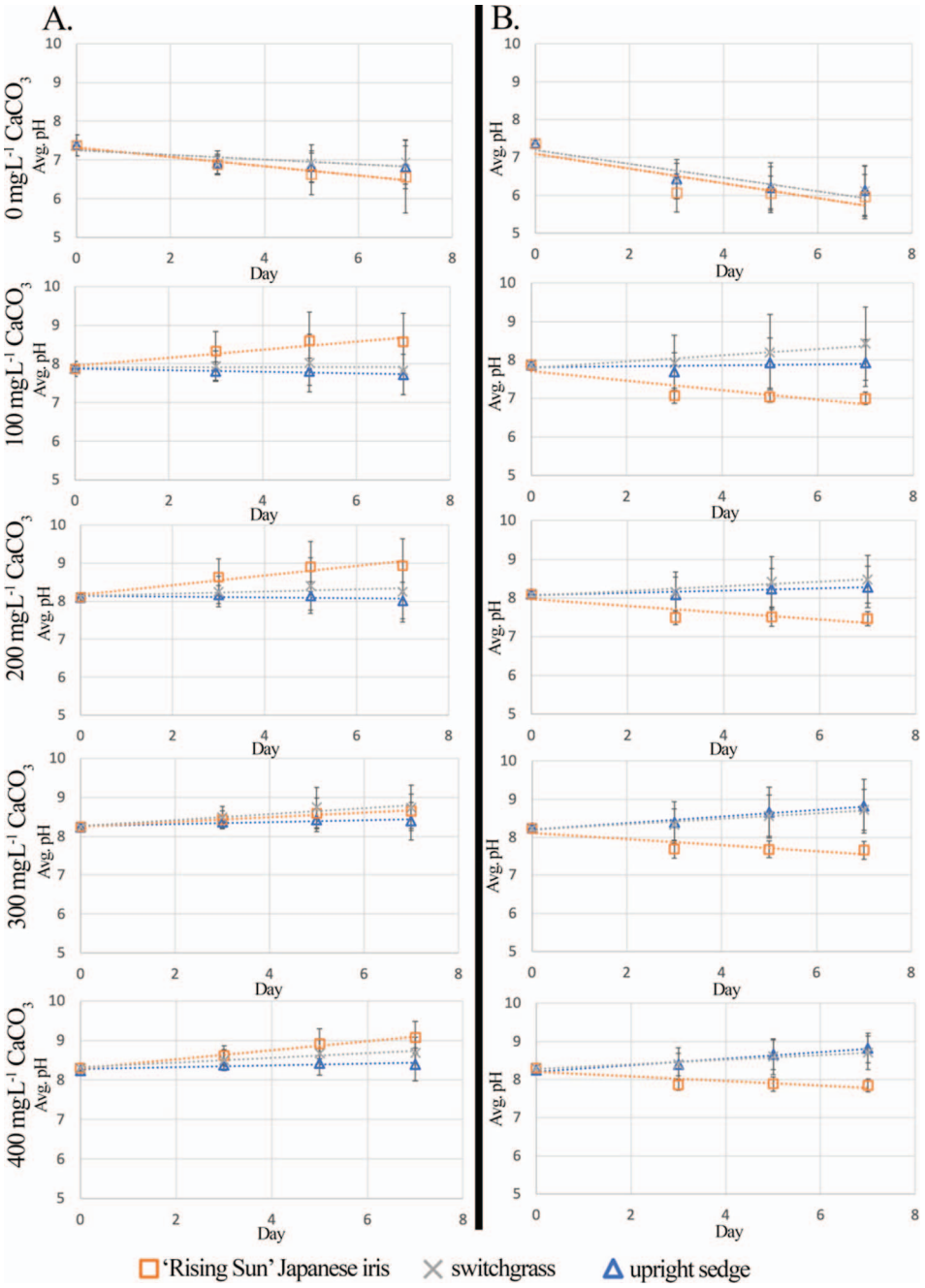

Fig. 2. The pH on Days 0, 3, 5, and 7 averaged (n=18) over 6-weeks during (A) 2016 and (B) 2017 as affected by the presence of three plant species ('Rising Sun' Japanese iris, switchgrass, and upright sedge) and five alkalinity levels (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$ using sodium bicarbonate).

0.75 units over 7 days (Fig. 2A). Conversely, in 2017, Japanese iris reduced solution pH on average by 0.58 ± 0.24 pH units over 7 days (Fig. 2B). Explanation of the differences between the two years is likely linked to plant growth and development, as Japanese iris grew poorly in 2016 compared with 2017. Other plants, including rapeseed (*Brassica napus* L.), ryegrass (*Lolium* spp. Lam.), and maize (*Zea mays* L.) have demonstrated enhanced release of either protons or organic anions, reducing pH, in response to nutritional limitations within the environment (Bertrand et al. 1999, Neumann and Römheld 1999). Our findings in 2017 align with previous findings, in which Japanese iris also decreased the pH of the water column on average by 0.3 pH units compared to other species of plants (Garcia Chance et al. 2019). Acidification of the root zone via release of organic acids by Japanese iris roots was posited as an explanation for the change. Species of Iris have been documented to contain carboxylic acids within its leaves and rhizomes that can be released during growth (Mikhailenko et al. 2018).

Alkalinity effect on plant-aided nutrient remediation. The mean cumulative N and P removal for 2016 and 2017 by species are presented in Table 2 and Table 3. During 2016, total load reduction ranged from 2.6 to 54% for N and 4.6 to 62% for P across all species. In 2016, the greatest total

Table 2. Nitrogen mass balance calculations for 'Rising Sun' Japanese iris, switchgrass, and upright sedge across 5 alkalinity treatments (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$ using sodium bicarbonate) for two years (2016 and 2017) after 6-week exposure to nutrients in floating treatment wetlands. Values presented are the means (standard error) of the mean.

Rising Sun' Japanese iris	Total Nitrogen									
	2016					2017				
	Alkalinity level (mg·L^{-1})									
Mass Balance	**0**	**100**	**200**	**300**	**400**	**0**	**100**	**200**	**300**	**400**
Total Influent Load[z] (g·m^{-3}·experiment^{-1})	37.3 ± 0.54	33.8 ± 0.66	34.0 ± 0.30	39.3 ± 0.72	37.8 ± 1.37	36.4 ± 0.36	39.3 ± 2.39	32.4 ± 0.55	41.3 ± 1.43	38.6 ± 1.04
Total Effluent Load[y] (g·m^{-3}·experiment^{-1})	34.5 ± 1.52	29.1 ± 1.35	25.4 ± 1.99	38.3 ± 1.84	36.5 ± 1.95	8.90 ± 1.89	8.70 ± 1.39	9.20 ± 1.05	8.40 ± 1.74	12.1 ± 1.94
Total Load Reduction[x] (g·m^{-3}·experiment^{-1})	*2.85*	*4.72*	*8.61*	*1.00*	*1.29*	*27.5*	*30.7*	*23.2*	*32.8*	*26.5*
% Load Reduction	7.64%	14.0%	25.3%	2.56%	3.42%	75.5%	77.9%	71.6%	79.5%	68.7%
Plant Uptake[w] (g·experiment^{-1})	*1.06*	*0.75*	*1.42*	*0.50*	*0.60*	*0.96*	*0.72*	*1.03*	*1.23*	*0.97*
% Plant Uptake Contribution	37.1%	16.0%	16.5%	49.4%	46.6%	3.50%	2.36%	4.46%	3.74%	3.64%
Other Removal Processes	1.80	3.96	7.19	0.51	0.69	26.5	29.9	22.2	31.6	25.5
Switchgrass	**2016**					**2017**				
	Alkalinity level (mg·L^{-1})									
Mass Balance	**0**	**100**	**200**	**300**	**400**	**0**	**100**	**200**	**300**	**400**
Total Influent Load[z] (g·m^{-3}·experiment^{-1})	37.3 ± 0.54	33.8 ± 0.66	34.0 ± 0.30	39.3 ± 0.72	37.8 ± 1.37	36.4 ± 0.36	39.3 ± 2.39	32.4 ± 0.55	41.3 ± 1.43	38.6 ± 1.04
Total Effluent Load[y] (g·m^{-3}·experiment^{-1})	19.9 ± 2.58	24.4 ± 2.17	18.6 ± 1.57	25.0 ± 1.70	31.1 ± 1.30	40.3 ± 1.14	27.7 ± 1.39	21.5 ± 1.63	33.8 ± 1.57	31.0 ± 1.83
Total Load Reduction[x] (g·m^{-3}·experiment^{-1})	*17.4*	*9.41*	*15.4*	*14.3*	*6.70*	*-3.85*	*11.7*	*10.9*	*7.43*	*7.62*
% Load Reduction	46.7%	27.9%	45.2%	36.4%	17.7%	-10.6%	29.6%	33.7%	18.0%	19.8%
Plant Uptake[w] (g·experiment^{-1})	*1.01*	*1.54*	*1.04*	*1.34*	*1.25*	*0.13*	*0.22*	*0.19*	*0.23*	*0.20*
% Plant Uptake Contribution	5.81%	16.4%	6.80%	9.36%	18.6%	-3.40%	1.86%	1.70%	3.05%	2.59%
Other Removal Processes	16.43	7.87	14.32	12.95	5.45	-3.98	11.44	10.74	7.21	7.42
Upright sedge	**2016**					**2017**				
	Alkalinity level (mg·L^{-1})									
Mass Balance	**0**	**100**	**200**	**300**	**400**	**0**	**100**	**200**	**300**	**400**
Total Influent Load[z] (g·m^{-3}·experiment^{-1})	37.3 ± 0.54	33.8 ± 0.66	34.0 ± 0.30	39.3 ± 0.72	37.8 ± 1.37	36.4 ± 0.36	39.3 ± 2.39	32.4 ± 0.55	41.3 ± 1.43	38.6 ± 1.04
Total Effluent Load[y] (g·m^{-3}·experiment^{-1})	17.3 ± 1.26	30.9 ± 1.53	24.1 ± 1.46	24.3 ± 1.32	28.8 ± 1.78	26.6 ± 1.59	26.6 ± 1.45	23.1 ± 1.27	18.8 ± 1.85	28.3 ± 1.28
Total Load Reduction[x] (g·m^{-3}·experiment^{-1})	*20.1*	*2.85*	*9.89*	*15.0*	*9.02*	*9.82*	*12.7*	*9.35*	*22.4*	*10.3*
% Load Reduction	53.7%	8.4%	29.1%	38.1%	23.9%	26.9%	32.3%	28.8%	54.4%	26.7%
Plant Uptake[w] (g·experiment^{-1})	*1.29*	*0.97*	*0.47*	*1.11*	*1.29*	*0.27*	*0.28*	*0.35*	*0.19*	*0.26*
% Plant Uptake Contribution	6.45%	34.0%	4.73%	7.41%	14.3%	2.78%	2.17%	3.71%	0.84%	2.51%
Other Removal Processes	18.8	1.88	9.42	13.9	7.73	9.55	12.4	9.00	22.3	10.0

[z]Total influent load = Summation of average nutrient influent at day 0 (fill) over the six-week experiment by volume of the experimental unit

[y]Total effluent load = Summation of average nutrient influent at day 7 (final day of 7-day HRT) over the six-week experiment by volume of the experimental unit

[x]Total load reduction = Difference between total influent and total effluent

[w]Plant Uptake = Nutrient concentration of plant (one per experimental unit) multiplied by dry mass then multiplied by 3 (representative of the number of plants per experimental unit)

Table 3. Phosphorus mass balance calculations for 'Rising Sun' Japanese iris, switchgrass, and upright sedge across 5 alkalinity treatments (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO^3$ using sodium bicarbonate) for two years (2016 and 2017) after 6-week exposure to nutrients in floating treatment wetlands. Values presented are the means (standard error) of the mean.

Rising Sun' Japanese iris	Total Phosphorus									
	2016					2017				
	Alkalinity level (mg·L^{-1})									
Mass Balance	**0**	**100**	**200**	**300**	**400**	**0**	**100**	**200**	**300**	**400**
Total Influent Load[z] (g·m^{-3}·experiment^{-1})	16.2 ± 0.29	15.9 ± 0.32	17.1 ± 0.13	16.5 ± 0.15	18.1 ± 0.56	19.2 ± 0.20	19.9 ± 0.92	20.4 ± 0.6	19.5 ± 0.75	16.5 ± 0.96
Total Effluent Load[y] (g·m^{-3}·experiment^{-1})	12.1 ± 0.44	14.8 ± 0.66	15.4 ± 0.52	14.8 ± 0.52	13.7 ± 0.63	7.33 ± 0.89	9.51 ± 1.27	9.50 ± 1.09	11.0 ± 0.97	12.7 ± 0.59
Total Load Reduction[x] (g·m^{-3}·experiment^{-1})	*4.09*	*1.09*	*1.69*	*1.67*	*4.46*	*5.92*	*5.21*	*5.44*	*4.25*	*1.90*
% Load Reduction	25.2%	6.87%	9.85%	10.1%	24.6%	61.8%	52.3%	53.4%	43.7%	23.0%
Plant Uptake[w] (g·experiment^{-1})	*0.11*	*0.06*	*0.11*	*0.07*	*0.06*	*0.12*	*0.11*	*0.12*	*0.14*	*0.10*
% Plant Uptake Contribution	2.69%	5.48%	6.52%	4.19%	1.34%	2.03%	2.11%	2.21%	3.30%	5.28%
Other Removal Processes	3.98	1.03	1.58	1.60	4.40	5.80	5.10	5.32	4.11	1.80
Switchgrass	**2016**					**2017**				
	Alkalinity level (mg·L^{-1})									
Mass Balance	**0**	**100**	**200**	**300**	**400**	**0**	**100**	**200**	**300**	**400**
Total Influent Load[z] (g·m^{-3}·experiment^{-1})	16.2 ± 0.29	15.9 ± 0.32	17.1 ± 0.13	16.5 ± 0.15	18.1 ± 0.56	19.2 ± 0.20	19.9 ± 0.92	20.4 ± 0.60	19.5 ± 0.75	16.5 ± 0.96
Total Effluent Load[y] (g·m^{-3}·experiment^{-1})	11.4 ± 0.70	13.8 ± 0.82	14.5 ± 0.97	15.1 ± 0.86	15.9 ± 0.51	15.1 ± 0.70	14.2 ± 0.84	14.8 ± 0.79	15.2 ± 1.09	13.0 ± 0.96
Total Load Reduction[x] (g·m^{-3}·experiment^{-1})	*4.85*	*2.14*	*2.61*	*1.40*	*2.27*	*4.02*	*5.70*	*5.61*	*4.28*	*3.42*
% Load Reduction	29.9%	13.4%	15.2%	8.48%	12.5%	21.0%	28.6%	27.6%	22.0%	20.8%
Plant Uptake[w] (g·experiment^{-1})	*0.12*	*0.14*	*0.13*	*0.14*	*0.11*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*
% Plant Uptake Contribution	2.48%	6.55%	4.99%	10.0%	4.84%	0.25%	0.18%	0.18%	0.23%	0.29%
Other Removal Processes	4.73	2.00	2.48	1.26	2.16	4.01	5.69	5.60	4.27	3.41
Upright sedge	**2016**					**2017**				
	Alkalinity level (mg·L^{-1})									
Mass Balance	**0**	**100**	**200**	**300**	**400**	**0**	**100**	**200**	**300**	**400**
Total Influent Load[z] (g·m^{-3}·experiment^{-1})	16.2 ± 0.29	15.9 ± 0.32	17.1 ± 0.13	16.5 ± 0.15	18.1 ± 0.56	19.2 ± 0.20	19.9 ± 0.92	20.4 ± 0.60	19.5 ± 0.75	16.5 ± 0.96
Total Effluent Load[y] (g·m^{-3}·experiment^{-1})	12.0 ± 0.55	15.2 ± 0.59	16.1 ± 0.84	15.1 ± 0.76	15.9 ± 0.54	13.8 ± 0.63	15.3 ± 0.80	14.7 ± 0.98	13.1 ± 1.10	13.9 ± 0.72
Total Load Reduction[x] (g·m^{-3}·experiment^{-1})	*4.23*	*0.74*	*1.06*	*1.40*	*2.27*	*5.39*	*4.63*	*5.63*	*6.32*	*2.53*
% Load Reduction	26.1%	4.64%	6.19%	8.48%	12.5%	28.1%	23.2%	27.6%	32.5%	15.4%
Plant Uptake[w] (g·experiment^{-1})	*0.12*	*0.11*	*0.06*	*0.10*	*0.12*	*0.03*	*0.03*	*0.02*	*0.01*	*0.02*
% Plant Uptake Contribution	2.84%	14.9%	5.66%	7.16%	4.84%	0.56%	0.65%	0.36%	0.16%	0.79%
Other Removal Processes	4.11	0.63	1.00	1.30	2.16	5.36	4.60	5.61	6.31	2.51

[z]Total influent load = Summation of average nutrient influent at day 0 (fill) over the six-week experiment by volume of the experimental unit

[y]Total effluent load = Summation of average nutrient influent at day 7 (final day of 7-day HRT) over the six-week experiment by volume of the experimental unit

[x]Total load reduction = Difference between total influent and total effluent

[w]Plant Uptake = Nutrient concentration of plant (one per experimental unit) multiplied by dry mass then multiplied by 3 (representative of the number of plants per experimental unit)

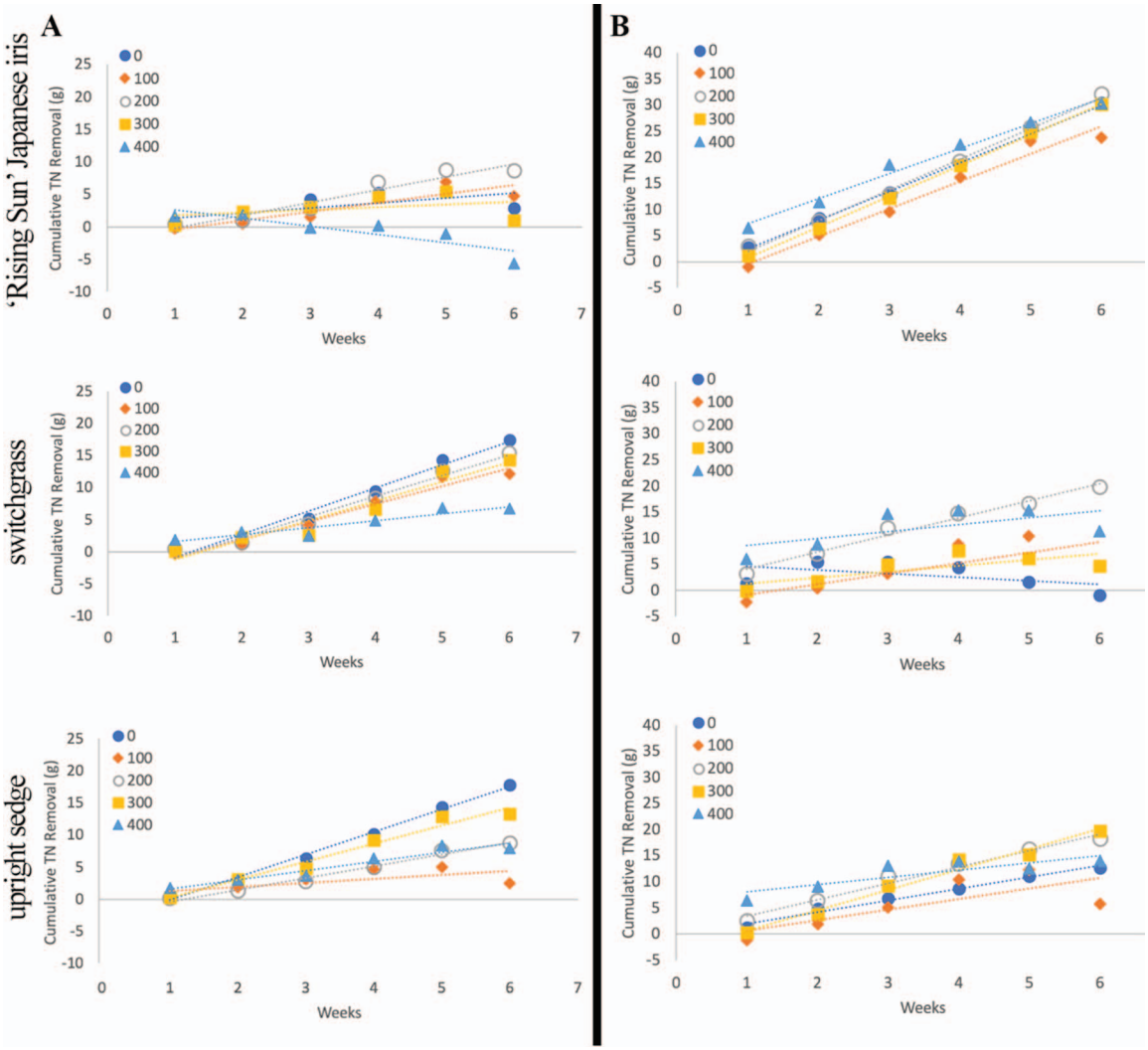

Fig. 3. Mean cumulative removal of total nitrogen during the (A) 2016 and (B) 2017 studies by alkalinity treatment (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$ using sodium bicarbonate) and plant (upright sedge, switchgrass, and 'Rising Sun' Japanese iris). Fitted linear regression lines represent removal rates over the 6-week experiment duration. Cumulative removal was calculated by averaging g·m^{-3}·experiment^{-1} values from 3 experimental units and adding average values each week for a total of 6 weeks.

removal of N and P across all species occurred in 0 mg·L^{-1} $CaCO_3$ treatments (13.5 ± 1.79 g·m^{-3}· N and 4.39 ± 0.56 g·m^{-3}· P; $p \leq 0.05$). FTWs planted with switchgrass and upright sedge removed more N than Japanese iris ($p \leq 0.05$) and FTWs planted with switchgrass removed more P than the other two plant species ($p \leq 0.05$). During 2017, total load reduction ranged from -11 to 80% for N and 15 to 62% for P across all plant species. In 2017, contrary to 2016 results, FTWs established with Japanese iris removed more N and P than all other treatments (Tables 2 & 3). Removal of N and P were similar among the two other species in 2017 ($p > 0.05$).

Japanese iris removed 60% more N in 2017 than in 2016 ($p < 0.001$). Differences in results could have been influenced by the health of Japanese iris during the 2016 season; while no observed disease or pest was present during this study, the health of Japanese iris from the same source used for other concurrent studies conducted in 2016 suffered as a result of Iris borer (*Macronoctua onusta* Grote). Iris borer often tunnel into the rhizome, especially during the late summer, and feed on the rhizome. The duration of the concurrent studies in 2016 differed (6 weeks and 16 weeks), so visual symptoms of Iris borer infestation may not have been evident as the plants were visually healthy, though growth effects such as reduced nutrient uptake were quantified after the experiment concluded. In the longer duration study, plant health was negatively impacted, leading to detection and determination of the species of pest present. Japanese iris effectively reduced N and P levels during 2017, suggesting it is promising for use over the range of alkalinity levels, with total load reduction similar across alkalinity treatments ($p > 0.05$).

The cumulative N and P removals during the 6-week study for both 2016 and 2017 are shown in Figures 3 & 4. Linear trend lines were fitted through the mean cumulative removal over time, and the slope of these trend lines represent the removal rate in g·week^{-1} for each treatment and species (Table 4). In 2016, N and P removal rates were highest for upright sedge in 0 mg·L^{-1} $CaCO_3$ with 3.95 g·week^{-1} N and 0.93 g·week^{-1} P ($p < 0.05$; Fig. 3 and Table 4). However, for Japanese iris, N removal rates were

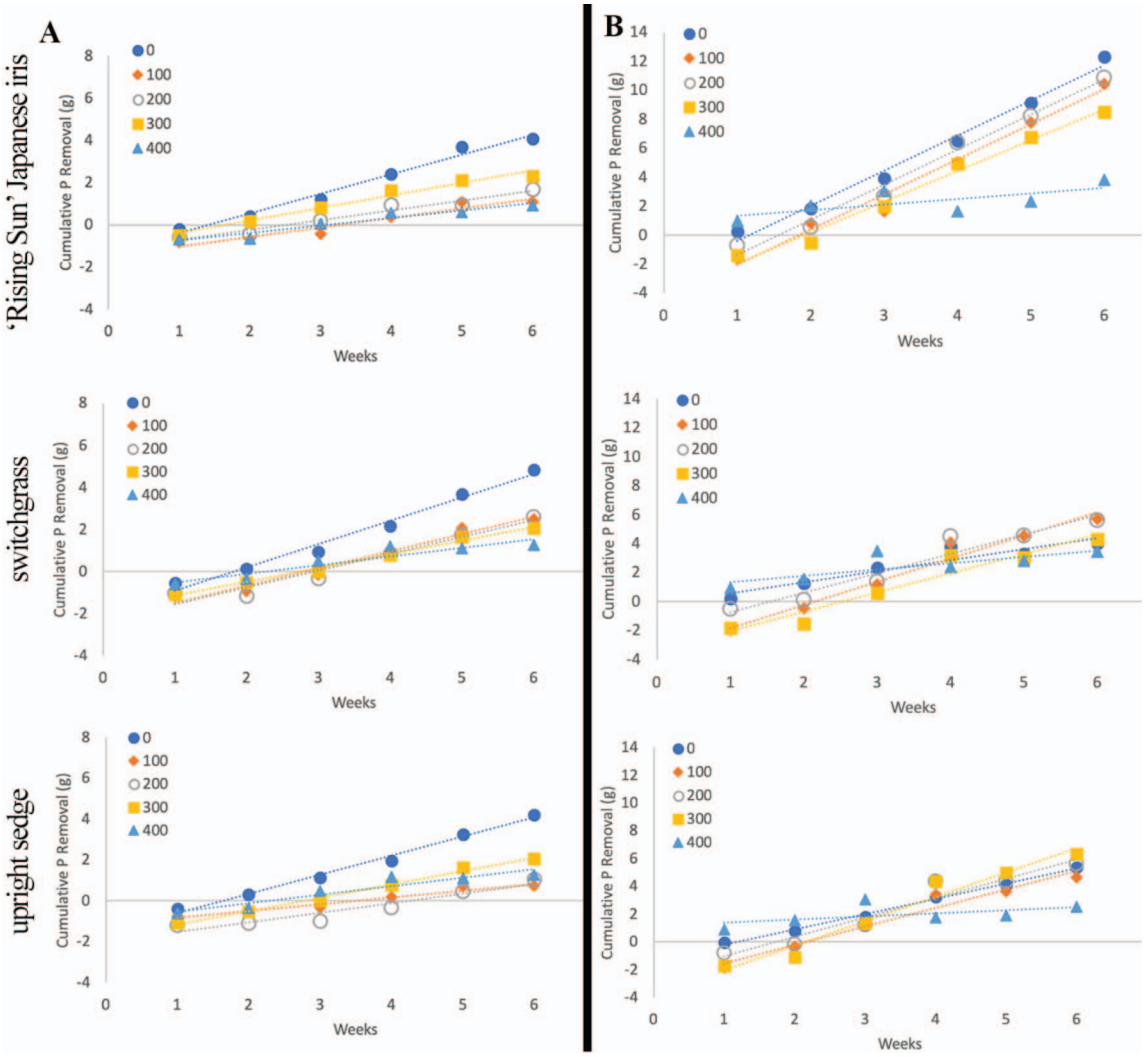

Fig. 4. Mean cumulative removal of phosphorus during the (A) 2016 and (B) 2017 studies by alkalinity treatment (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$ using sodium bicarbonate) and plant (upright sedge, switchgrass, and 'Rising Sun' Japanese iris). Fitted linear regression lines represent removal rates over the 6-week experiment duration. Cumulative removal was calculated by averaging g·m^{-3}·experiment^{-1} values from 3 experimental units and adding average values each week for a total of 6 weeks.

greatest in the 100 and 200 mg·L^{-1} $CaCO_3$ treatments ($p < 0.05$). Switchgrass N removal rates were similar for 0, 100, 200, and 300 mg·L^{-1} $CaCO_3$ treatments ($p > 0.05$; Fig. 3 and Table 4).Greatest P removal rates occurred in 0 mg·L^{-1} $CaCO_3$ treatments for both Japanese iris and switchgrass ($p < 0.05$).

The 2017 N ($p < 0.001$) and P ($p < 0.05$) removal rates were greater than those in 2016 for Japanese iris. In fact, Japanese iris showed the greatest removal rate constants for both N and P among the three species (Fig. 4). Japanese iris N and P removal rates were similar across all alkalinity treatments, except for the 400 mg·L^{-1} $CaCO_3$ treatment for P (avg. 5.47 ± 0.46 g·m^{-3}·week^{-1} of N removed and 1.95 ± 0.89 g·m^{-3}·week^{-1} of P removed; $p > 0.05$ for all treatments except P in 400 mg·L^{-1} $CaCO_3$). Removal rates for Japanese iris in 2017 were higher than those found for other studies. Spangler et al. (2019) calculated removal rates of 3.81 g·m^{-2}·week^{-1} of N removed and 1.17 g·m^{-2}·week^{-1} of P removed and Iamchaturapatr et al. (2007) found removal rates of 2.80 g·m^{-2}·week^{-1} of N removed and 1.82 g·m^{-2}·week^{-1} of P for Japanese iris. In comparison to literature values, removal rates for both switchgrass and upright sedge were lower in 2017. The lower removal rates obtained in our study could be attributed to a number of factors, including stress associated with exposure to higher alkalinity and Na levels.

Plant tissue accumulation of N and P over each 6-week experiment as part of the removal process are detailed in Tables 2 and 3 as well as average mass per plant in Figure 5. The treatment combinations of plant species and alkalinity level did not impact plant accumulation of N or P in 2016 ($p = 0.54$ for N and $p = 0.22$ for P). No difference between plant species was evident with regard to P uptake in 2016 ($p = 0.06$). Cumulatively, across the 6-week experiment in 2016 in comparison to switchgrass and upright sedge, Japanese iris accumulated the least amount of N with an average 0.87 ± 0.37 g N ($p = 0.007$, Table 3). Similar to 2016, treatment combinations did not impact N and P plant uptake in 2017 ($p = 0.24$ for N and $p = 0.66$ for P; Fig. 5). However, Japanese iris accumulated more N and P in its tissues than did switchgrass and upright sedge ($p < 0.01$ for both N and P, Fig. 5). Cumulatively, across the

Table 4. The mean rate of nitrogen (N) and phosphorus (P) loads ($g \cdot m^{-2} \cdot week^{-1}$) removed and associated correlation (load × time) statistics (R^2) assessing variability explained by the linear regression model fit for floating treatment wetland studies (2016 and 2017) for five alkalinity levels and three plant species (n=12).

Treatment	Rising Sun' Japanese iris		Switchgrass		Upright sedge	
2016	**N ($g \cdot week^{-1}$)**	**N (R^2)**	**N ($g \cdot week^{-1}$)**	**N (R^2)**	**N ($g \cdot week^{-1}$)**	**N (R^2)**
0 $mg \cdot L^{-1}$ $CaCO_3$	0.76	0.44	3.60	0.98	3.95	0.99
100 $mg \cdot L^{-1}$ $CaCO_3$	1.36	0.79	2.79	0.97	0.71	0.44
200 $mg \cdot L^{-1}$ $CaCO_3$	1.95	0.92	3.21	0.97	2.07	0.98
300 $mg \cdot L^{-1}$ $CaCO_3$	0.42	0.15	3.02	0.93	3.18	0.97
400 $mg \cdot L^{-1}$ $CaCO_3$	-1.27	0.77	1.07	0.87	1.61	0.93
	P ($g \cdot week^{-1}$)	**P (R^2)**	**P ($g \cdot week^{-1}$)**	**P (R^2)**	**P ($g \cdot week^{-1}$)**	**P (R^2)**
0 $mg \cdot L^{-1}$ $CaCO_3$	0.93	0.98	1.11	0.98	0.93	0.99
100 $mg \cdot L^{-1}$ $CaCO_3$	0.45	0.93	0.81	0.97	0.33	0.96
200 $mg \cdot L^{-1}$ $CaCO_3$	0.46	0.96	0.80	0.95	0.47	0.91
300 $mg \cdot L^{-1}$ $CaCO_3$	0.59	0.98	0.65	0.99	0.65	0.99
400 $mg \cdot L^{-1}$ $CaCO_3$	0.35	0.93	0.41	0.88	0.41	0.88
2017	**N ($g \cdot week^{-1}$)**	**N (R^2)**	**N ($g \cdot week^{-1}$)**	**N (R^2)**	**N ($g \cdot week^{-1}$)**	**N (R^2)**
0 $mg \cdot L^{-1}$ $CaCO_3$	5.49	2.23	-0.69	0.99	0.98	0.24
100 $mg \cdot L^{-1}$ $CaCO_3$	5.27	2.03	2.02	0.97	0.57	0.61
200 $mg \cdot L^{-1}$ $CaCO_3$	5.87	3.16	3.27	0.99	0.98	0.98
300 $mg \cdot L^{-1}$ $CaCO_3$	5.90	3.91	1.14	0.99	0.98	0.56
400 $mg \cdot L^{-1}$ $CaCO_3$	4.80	1.40	1.34	0.98	0.74	0.42
	P ($g \cdot week^{-1}$)	**P (R^2)**	**P ($g \cdot week^{-1}$)**	**P (R^2)**	**P ($g \cdot week^{-1}$)**	**P (R^2)**
0 $mg \cdot L^{-1}$ $CaCO_3$	2.42	0.99	0.76	0.89	1.10	0.99
100 $mg \cdot L^{-1}$ $CaCO_3$	2.42	0.98	1.61	0.97	1.33	0.96
200 $mg \cdot L^{-1}$ $CaCO_3$	2.42	0.98	1.34	0.93	1.40	0.94
300 $mg \cdot L^{-1}$ $CaCO_3$	2.12	0.99	1.34	0.93	1.75	0.96
400 $mg \cdot L^{-1}$ $CaCO_3$	0.38	0.53	0.43	0.61	0.23	0.31

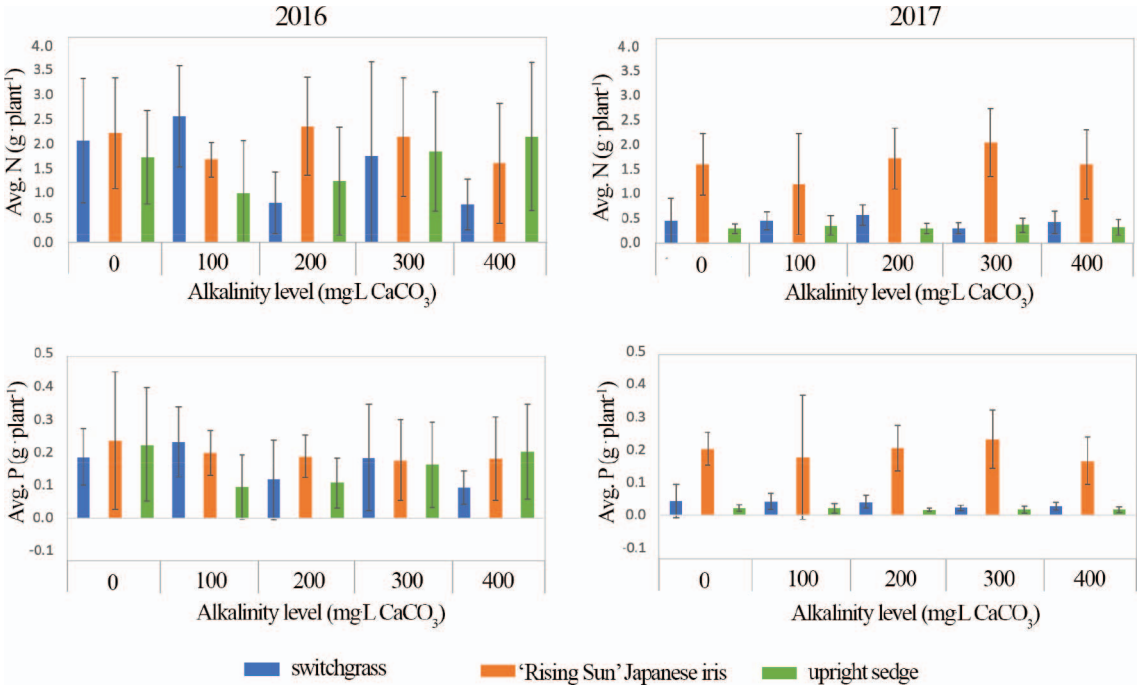

Fig. 5. Nitrogen and phosphorus mass accumulated on a per plant basis at the conclusion of the 2016 and 2017 studies. Each bar represents the mean ± standard error of nutrients accumulated within three plants harvested from separate experimental units planted with 'Rising Sun' Japanese iris, switchgrass, or upright sedge.

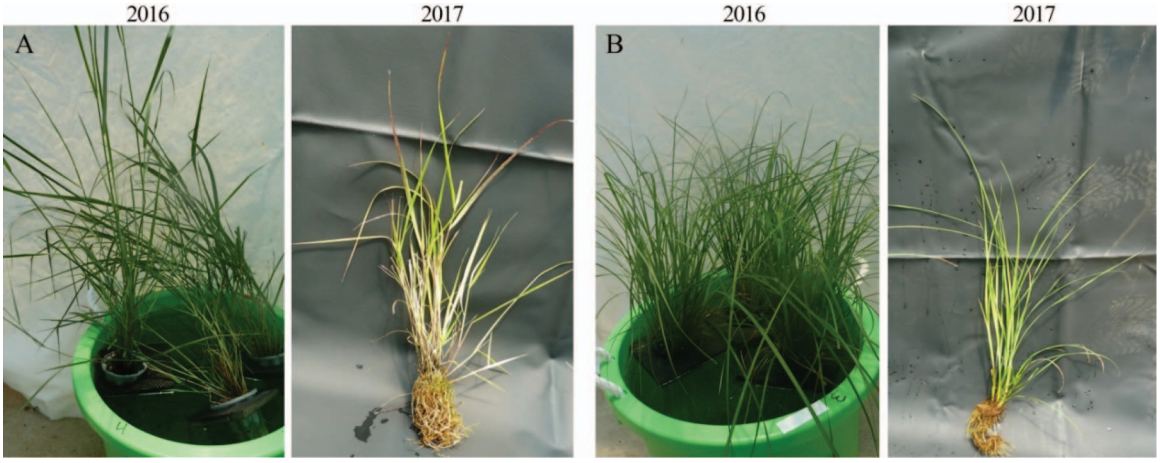

Fig. 6. Growth and visual comparison between 2016 and 2017 experimental studies for (A) switchgrass and (B) upright sedge. Each image is a representation of an average specimen per species.

6-week experiment in 2017, Japanese iris accumulated the greatest amount of both N and P with an average 0.98 ± 0.18 g N and 0.12 ± 0.01 g P ($p = 0.007$, Table 3).

While the mass of N and P fixed in the tissues of Japanese iris was greater in 2017 compared to 2016 ($p < 0.01$ for N and P), N and P mediated removal by upright sedge and switchgrass was much lower in 2017 ($p < 0.05$ for all; Table 3). Overall, growth of upright sedge and switchgrass decreased while chlorosis increased in 2017 compared to 2016 (Table 1; Fig. 6). Reduced growth was most evident in the 0 $mg \cdot L^{-1}$ $CaCO_3$ treatment, as plant growth and dry mass were lower in 2017 than in 2016 ($p < 0.001$), subsequently N removal was also diminished in 2017 in comparison with 2016 (Fig. 3). While plant species remained consistent between the two years, the source of plants changed from Supplier B to C due to lack of plant supply in 2017 by supplier B. Of note, Supplier B is located in the northeast, USDA hardiness zone 6a, while Supplier C is located in the southeast, USDA hardiness zone 8a. Therefore, when plants were shipped in early July, the relative age of the plants and stage of their growth cycle likely differed, with plants from Supplier C potentially breaking dormancy earlier than plants from Supplier B. Therefore, while overall plant health could have been responsible for differences in growth and nutrient remediation of the plants, the relative age of the plant may have impacted plant alkalinity tolerance (Valdez-Aguilar and Reed 2007).

Despite the decrease in uptake of N and P by upright sedge and switchgrass in 2017, total load reduction of P was higher for both species in 2017 ($p < 0.01$ for both), and similar to 2016 for N total load reduction in upright sedge ($p = 0.23$; Table 2). This incongruity could partially be explained by algal blooms witnessed within the systems in 2017, specifically in upright sedge and switchgrass tubs, potentially because the poor growth and nutrient removal of the plants allowed an excess supply of nutrients for algal production. Jones et al. (2017) found algal growth to be in excess of 80% in open systems compared to systems with vigorous plant systems. Algal blooms were also present in the control (no plant present) tubs and thus relative comparison of remediation values when no plants were present with points in time when plants were present allow us to begin to quantify the contribution of algal communities to nutrient remediation within these systems. In 2017, total load reduction by the control, upright sedge, and switchgrass tubs were similar for both N ($p = 0.07$ switchgrass and $p = 0.14$ sedge) and P ($p = 0.43$ switchgrass and $p = 0.56$ sedge), while Japanese iris nutrient load reduction was greater ($p < 0.001$ N and $p = 0.003$ P). Chlorophyll readings were not taken and are recommended for future studies so that the influence of algal communities on nutrient remediation can be accounted for in a more quantitative manner.

Overall contribution of plant uptake to the load reduction ranged from 0.84 to 49.4% for N and 0.18 to 14.9% for P across both studies (Table 2 and 3). Plant uptake contribution to nutrient remediation leaves an average load reduction of 8.19 ± 5.96 g N and 2.29 ± 1.36 g P unaccounted for in 2016 and 15.7 ± 10.2 g N and 4.63 ± 1.29 g P in 2017 (Tables 2 and 3). Load reduction not attributable to plant uptake alone is common within wetland systems and could be attributable to denitrification and loss of N from the system through N_2 volatilization, P sorption processes (absorption and adsorption), or presence of other organic matter within the system including weeds, algae, or microbial communities unaccounted for in the plant harvest process. Borin and Salvato (2012) found plant removal accounted for 53-75% of their N load, microbial communities accounted for 0.4-4.9%, and gaseous losses were estimated to be 17-37% of the total load. Their plant removal contribution was higher than that found within this study, which more closely aligns with results found in the literature, 0.7%-24.0% N removal (Garcia Chance and White 2018, Gottschall et al. 2007, Lin et al. 2002, Zhang et al. 2017). Differences in plant contribution to N load reduction could be attributed to the form of nitrogen supplied, nutrient concentration, water temperature, evapotranspiration, duration of experiments, and alkalinity levels (Borin and Salvato 2012, White and Cousins 2013).

Impact of sodium. Plant uptake of Na^+, K^+, and Ca^+ are provided in mg· $plant^{-1}$ for the 2016 and 2017 studies in Figure 7. In 2016, the excess Na^+ in solution did not

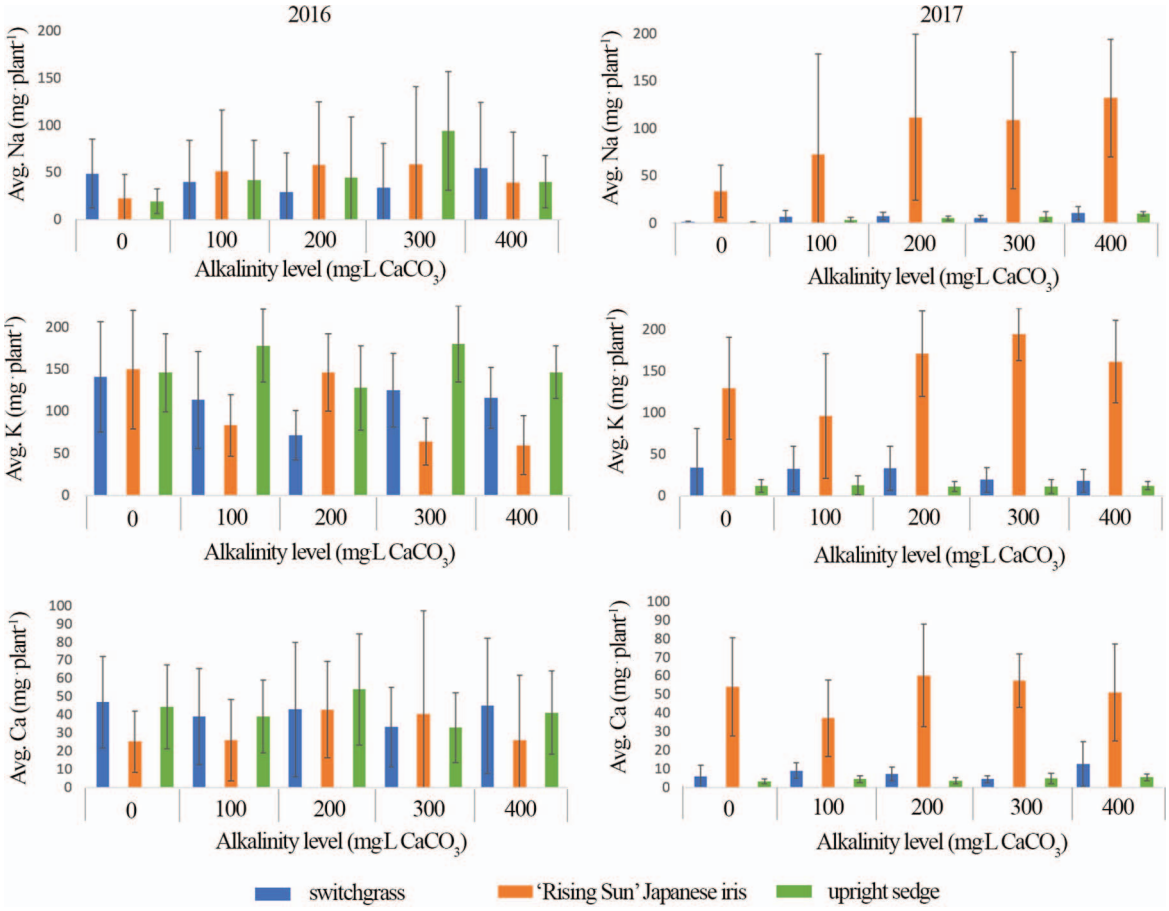

Fig. 7. Sodium (Na^+), potassium (K^+), and calcium (Ca^+) mass accumulated on a per plant basis at the conclusion of the 2016 and 2017 studies. Each bar represents the mean ± standard error of nutrients accumulated within three plants harvested from separate experimental units planted with 'Rising Sun' Japanese iris, switchgrass, or upright sedge.

influence the mass of Na^+ accumulated within plant tissue, regardless of alkalinity treatment ($p = 0.62$) or plant species ($p = 0.91$). While the concentration of Na^+ in solution increased as alkalinity increased, the presence of excess Na^+ did not negatively influence plant cation uptake or exchange. In 2017, the mass of Na^+ accumulated in 'Rising Sun' Japanese iris was greater than that of the other two species ($p < 0.001$), but Na^+ uptake was similar across alkalinity treatments ($p = 0.62$). Traditionally, increases in Na^+ are associated with the adverse effects of Na^+ toxicity, including depression of photosynthesis and plant growth (Munns and Tester 2008, Horie et al. 2012, Deinlein et al. 2014, Maathuis 2014, Hanin et al. 2016). An increase in Na^+ content is typically accompanied by K^+ and Ca^+ loss in plants exposed to salt (NaCl) stress. Potassium is an essential macronutrient in plants, generally comprising 4 to 6% of a plants dry mass and is recognized as a rate-limiting factor for crop yield and quality (Dreyer and Uozumi 2011, Zorb et al. 2014). K^+ plays an important role in plant response to both biotic (disease and pests) and abiotic stresses such as drought, salinity, cold, and waterlogging (Wang et al. 2013b, Shabala and Pottosin 2014). Alkalinity levels did not affect K^+ uptake in either the 2016 ($p = 0.74$) or 2017 ($p = 0.36$) studies (Fig. 7). Instead, a positive correlation between Na^+ and K^+ uptake was found in 2016 ($R = 0.78$) and in Japanese iris in 2017 ($R = 0.84$), with no difference between plant species in 2016 ($p = 0.72$).

Calcium is another important cation that is commonly suppressed in high Na^+ solutions, affecting cell shape and size, photosynthesis, and water transport among other impacts (Cabot et al. 2009). Assessing the mass of Ca^+ accumulated in plant tissues resulted in similar findings to K^+, as alkalinity did not affect Ca^+ uptake in 2016 ($p = 0.77$) or 2017 ($p = 0.62$), although plant differences in 2017 ($p < 0.001$) showed a greater accumulation in Japanese iris in comparison to the other two species (Fig. 7). Regression plots of Ca^+ and Na^+ indicate a weak positive correlation in 2016 ($R = 0.54$, data not shown) and but no correlation between Ca^+ and Na^+ uptake in 2017 ($R = 0.24$, data not shown).

The mass of Na^+ in plant tissues resulting in inhibition of absorption and transport of cations varies greatly depending on the plant species and the growing conditions (Barbieri et al. 2012, Negrão et al. 2017). Therefore, it is difficult to determine if the accumulated Na^+ masses (1.1 to 130 mg·plant^{-1} Na^+) were great enough to negatively affect plant health. Some studies have reported only weak Na^+ effects on plant nutrient uptake (Epstein 1961, Epstein et al. 1963), or even stimulation of K^+ and Ca^+ influx by excess Na^+ (Rubio et al. 1995, Spalding et al. 1999), but such studies are in the minority. At micromolar Na^+

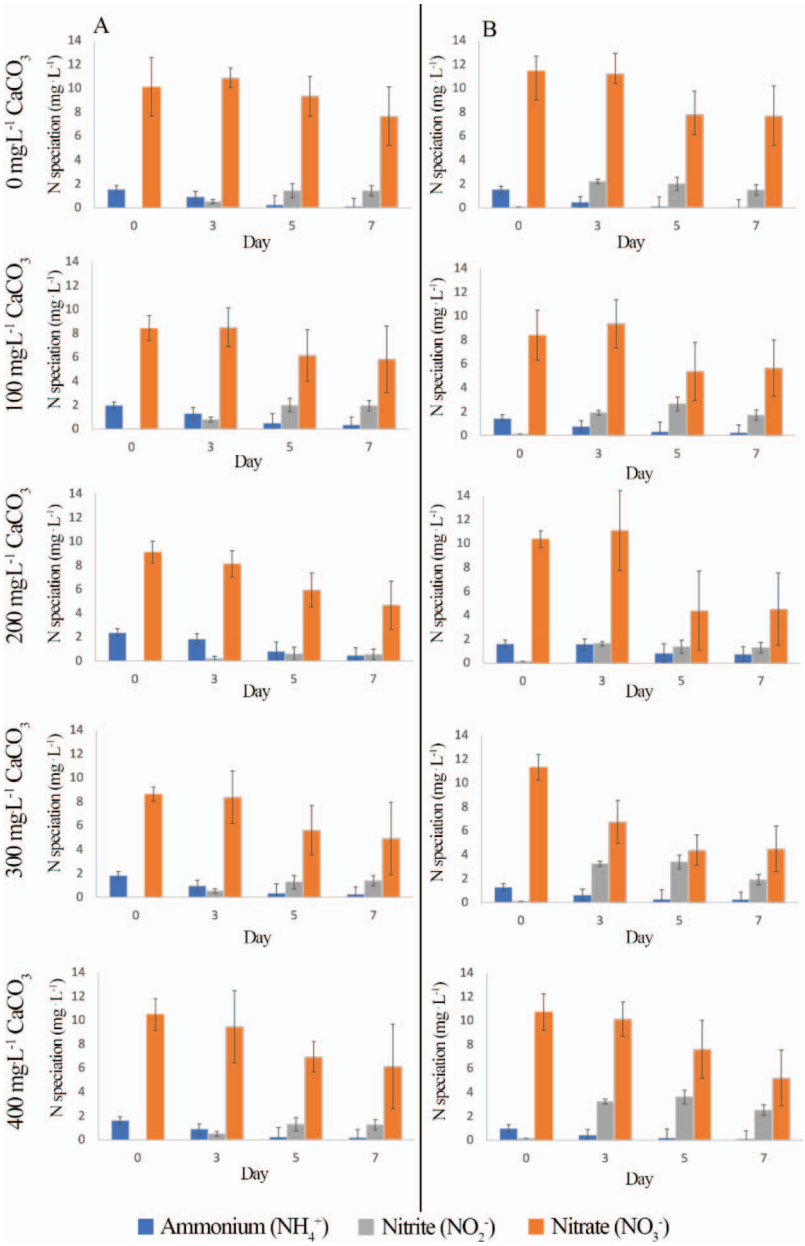

Fig. 8. Concentration of ammonium, nitrite, and nitrate remaining within the water column over a 7-day period as influenced by the presence of plants in a floating treatment wetland and alkalinity level (0, 100, 200, 300 and 400 mg·L^{-1} $CaCO_3$ using sodium bicarbonate) in A) 2016 and B) 2017. Fifty-four replicates per bar (6 sampling points X 9 EUs), each bar represents the mean ± standard error of the mean.

concentrations, K^+ uptake can be activated by the plant potassium transporter HKT1 (Garriga et al. 2017, Rubio et al. 1995). To further confound the role of Na^+ within this study, many studies on salinity stress and plant behavior assume that responses in hydroponic conditions, somewhat similar to experimental conditions, mimic those in soil. However, interactions between the soil solution and the soil matrix can affect responses to salinity stress not observed in aquatic systems (Tavakkoli et al. 2010a, Tavakkoli et al. 2010b).

Nitrogen speciation. Autotrophic bacteria facilitate nitrification, oxidizing ammonia to nitrite and, after that, nitrate. Because these bacteria are slow growing, nitrite tends to accumulate at significant concentrations in stagnant environments without water renewal (Hargreaves 2006). Given that our systems were static, with renewal occurring every seven days, it is possible that the conditions within the tubs tended toward nitrite accumulation, rather than complete denitrification. An analysis of the speciation of nitrogen (nitrite, nitrate, and ammonium) with the FTW tubs over the 6-week studies for each alkalinity level during 2016 and 2017 is shown in Figure 8. In 2016 and 2017, at Day 0 across all alkalinity treatments, the concentration of ammonium (2016 $p = 0.42$, 2017 $p = 0.68$) and nitrate (2016 $p = 0.48$, 2017 $p = 0.62$) present in solution were similar, so changes in speciation were not derived from initial differences. In both 2016 and 2017, the 200 mg·L^{-1} $CaCO_3$ treatment had the lowest nitrite concentration (0.35 ± 0.02 mg·L^{-1} 2016, $p < 0.001$ and

1.11± 0.03 mg·L^{-1} 2017, $p < 0.001$), followed by 0 mg·L^{-1} $CaCO_3$ (0.85 ± 0.02 mg·L^{-1} 2016, $p = 0.002$ and 1.44± 0.02 mg·L^{-1} 2017 $p < 0.001$). For 200 mg·L^{-1} $CaCO_3$, Day 7 nitrate levels were also the lowest compared to the other alkalinity treatment levels ($p < 0.01$, Fig. 8). Given this finding, paired with earlier data in which an increased N load reduction occurred in 200 mg·L^{-1} $CaCO_3$ for all plant species and both years (Table 2), it is possible that the nitrite within the system was fully transformed to nitrate and then taken up by the plants or removed by other processes within the FTW system (denitrification).

These results align with other research in stagnant microbial systems. According to Chen, et al. (2006), due to the possible stratification of alkalinity and pH in microbial communities, an alkalinity higher than 200 mg·L^{-1} $CaCO_3$ was recommended for denitrification processes, especially when the water renewal rate is minimal or nonexistent. However, Ebeling, et al. (2006) indicated that in systems with limited water exchange, alkalinity must be between 100 and 150 mg·L^{-1} $CaCO_3$ for denitrification. We did not find nitrite to nitrate transformation in 100 mg·L^{-1} $CaCO_3$ treatments, which could be due to one of several other factors that can affect the nitrification process, including the level of DO within the water column, carbon/nitrogen ratios, and temperature (Eberling et al. 2006). Unfortunately, dissolved oxygen levels were not measured during this experiment but would have provided insight into the nitrification process.

Many factors become confounding as the alkalinity of water changes, these include salinity, nutrient availability, algal growth, and the microbial activity of the system. As a result, the complexities of variable alkalinity scenarios may have large, unpredictable effects upon plant-based treatment systems, such as FTWs. Plant selection may impact the success of nutrient remediation aided by FTW systems, but plant-based factors other than plant species are also important to consider, such as time of transplant, relative age of the plant material, and preexisting infestation or infection by pests or diseases. Both experimental system design and the described plant-based factors were responsible for the variable nutrient remediation of the three species trialed during the 2016 and 2017 studies.

System alkalinity remained consistent following the initial adjustment. However, the presence of Japanese iris in FTW treatments resulted in changes to solution pH. In 2016 water pH was higher (+0.68) in the presence of Japanese iris in comparison to the other plants screened and lower in 2017 (-0.58). The reduction in N and P by Japanese iris was greater in 2017 than 2016, as was mass of N and P absorbed by the plants. Conversely, load reduction by upright sedge and switchgrass in 2017 was similar to or less than that in 2016, associated with a decrease in total mass fixed within the plant tissue. Differences in total load reduction and plant contributions could be attributed to algal blooms in tubs established with upright sedge and switchgrass in 2017. Algal communities aided nutrient removal in the system in addition to contributions of the macrophytes. While the concentration of Na^+ in solution increased as alkalinity increased, the presence of excess Na^+ did not negatively influence plant cation uptake or exchange. While P load reduction was greatest in the 0 mg·L^{-1} $CaCO_3$ treatment, N load reduction varied across alkalinity treatments. Stagnant systems, such as the ones utilized in this experiment, can slow the growth of autotrophic bacteria, allowing for an accumulation of nitrite, preventing oxidation of nitrite to nitrate. Some evidence of this occurred during both 2016 and 2017, as large concentrations of nitrite were detected in some treatments, reducing the availability of nitrate for plant uptake over the 7-day HRT. Overall, Japanese iris demonstrated consistent remediation both year to year as well as across each alkalinity treatment for both load reduction and nutrient accumulation within plant tissues. This may partially be due to the suspected capacity of 'Rising Sun' Japanese iris to alter the chemistry of its root zone and thus change pH or redox conditions, increasing availability or excluding nutrients as needed. Iris species warrant greater consideration and use in bioremediation systems, although both upright sedge and switchgrass could be utilized in FTWs established in ponds with appropriate alkalinity levels. Further work should consider assessing plants at different points within their growth cycle as well as expansion of the exposure times and decreased HRT.

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