Nutrient Recovery by Seven Aquatic Garden Plants in a Laboratory-scale Subsurface-constructed Wetland

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Abstract. Commercial nurseries use large amounts of water and nutrients to produce container-grown plants. The large volume of runoff containing nitrogen (N) and phosphorus (P) that leaves nurseries can contaminate surface and groundwater. Subsurface flow-constructed wetlands have been shown to effectively treat agricultural, industrial, and residential wastewater and to be well-suited for growers with limited production space. We investigated the possibility of using commercially available aquatic garden plants in subsurface-constructed wetlands to remove nutrients in a laboratory scale, gravel-based system. Seven popular aquatic garden plants received N and P from Hoagland's nutrient solution every 2 days for 8 weeks. These rates (0.39 to 36.81 mg·L⁻¹ of N and 0.07 to 6.77 mg·L⁻¹ P, respectively) encompassed low to high rates of nutrients found at various points between the discharge and inflow points of other constructed wetland systems currently in use at commercial nurseries. Plant biomass, nutrient recovery, and tissue nutrient concentration and content were measured. Whole plant dry weight positively correlated with total N and P supplied. Louisiana Iris hybrid 'Full Eclipse', *Canna* × *generalis* Bailey (pro sp.) 'Bengal Tiger', *Canna* × *generalis* Bailey (pro sp.) 'Yellow King Humbert', *Colocasia esculenta* (L.) Schott 'Illustris', *Peltandra virginica* (L.) Schott, and *Pontederia cordata* L. 'Singapore Pink' had the greatest N recovery rates. The P recovery rates were similar for the cannas, *Colocasia esculenta* 'Illustris', Louisiana Iris 'Full Eclipse', *Pe. virginica*, and *Po. cordata* 'Singapore Pink'. The potential exists for creating a sustainable nursery and greenhouse production system that incorporates a subsurface-constructed wetland planted with marketable horticultural crops that provide remediation and revenue.

Traditional production of container-grown plants involves the input of water, fertilizers, pesticides, and other agricultural

chemicals. Excessive leaching of nutrients and pesticides from containerized crops grown in soilless substrate may occur when

production is not managed appropriately (Schoene et al., 2006). The resulting runoff can be discharged from production areas and pollute surface and groundwater. Excess nutrients, notably nitrate-nitrogen (NO₃⁻-N) and soluble reactive phosphorus (PO₄³⁻, H₂PO₄⁻, H₂PO₄²⁻, and H₃PO₄⁻), encourage algal growth and accelerate eutrophication, primarily in freshwater systems. Also, high levels of nitrates in drinking water can cause methemoglobinemia in infants ("Blue Baby Syndrome"). To protect drinking water quality, the U.S. Environmental Protection Agency (EPA) mandates maximum allowable NO₃⁻-N contaminant levels in any discharged water at 10 mg·L⁻¹ (U.S. EPA, 1986). Federal limits on phosphorus (P) concentrations in freshwater have not been set, but the U.S. EPA recommends that total phosphates and total P levels not exceed 0.05 mg·L⁻¹ and 0.1 mg·L⁻¹, respectively (U.S. EPA, 1986). Greenhouse wastewater typically contains 100 mg·L⁻¹ NO₃⁻-N (Wood et al., 1999), whereas nursery runoff levels of NO₃⁻-N range from 0.1 to 135 mg·L⁻¹ (Alexander, 1993; Taylor et al., 2006; Yeager et al., 1993). A range of 0.01 to 20 mg·L⁻¹ P has been reported in nursery runoff (Alexander, 1993; Headley et al., 2001; Taylor et al., 2006).

The future of container nursery irrigation, according to 12 irrigation scientists, growers, and nursery organization directors, will be shaped by increasingly stringent regulations as many provisions of the 1972 Federal Clean Water Act are enforced (Beeson et al., 2004). Environmental concerns and regulatory pressure to reduce nutrient loadings in surface waters have led to the EPA enforcing its Total Maximum Daily Load (TMDL) program for all watersheds and bodies of water (U.S. EPA, 2000). Section 303(d)(1)(C) of the Clean Water Act defines the TMDL as the "level necessary to implement the applicable water quality standards." U.S. states are mandated to develop an appropriate TMDL for each water body and for each identified pollutant, which involves quantifying the total amount of pollutant loading a water body can receive from point and nonpoint sources and still maintain its designated use and value (e.g., drinking water, fish and wildlife habitat, and recreation). TMDLs of nutrients in agricultural runoff were recently adopted by environmental regulatory agencies in every state (Yeager, 2006). Nutrient-loading criteria for natural waters will eventually be established in every state. Furthermore, several states, including Maryland, Delaware, and California, have enacted nutrient management laws to control the quantity of fertilizer applied and to monitor the concentration of nutrients detected in nursery runoff (Beeson et al., 2004).

To comply with stricter environmental regulations, constructed wetlands have been promoted as a low-cost technology for reducing nutrient levels, pesticides, and other organic contaminants from nursery and greenhouse discharge water (Berghage et al., 1999; Fernandez et al., 1999). Two

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constructed wetland designs, surface flow and subsurface flow-constructed wetlands, are commonly used to treat agricultural wastewater (Berghage et al., 1999; Scholz and Lee, 2005). The large land area required by typical surface flow-constructed wetlands, which resemble natural wetlands, and the concomitant loss of production area has made them less suitable for greenhouse and nursery water treatment than subsurface flow-constructed wetlands (Berghage et al., 1999).

Subsurface flow-constructed wetlands consist of a lined or impermeable basin filled with a coarse medium having high hydraulic conductivity, typically gravel, and wetland plants (Kadlec and Knight, 1996). They can be operated in flow-through or batch treatment modes with varying hydraulic residence times (Burgoon et al., 1995). Removal or transformation of nitrogen (N) and P occurs through microbial assimilation/transformation, decomposition, plant uptake, adsorption-fixation, sedimentation, and volatilization (Brix and Schierup, 1989).

Plants have both dominant and supporting roles in N and P recovery. Besides the direct assimilation of N and P from wastewater, plant roots and rhizomes support microbial activity and facilitate microbial nitrification in gravel-based constructed wetlands (Gersberg et al., 1986; Huett et al., 2005). Their roots offer colonizing sites and exude carbohydrates, sugars, amino acids, enzymes, and many other compounds (Rovira, 1969). Certain plants oxidize the rhizosphere (Gersberg et al., 1986; Moorhead and Reddy, 1988), which also supports microbial growth and aids in the decomposition of organic matter.

Widely used aquatic emergent plants in subsurface flow-constructed wetland designs include reed canarygrass (*Phalaris arundinacea* L.), common reed [*Phragmites australis* (Cav.) Trin. ex Steud.], reed managrace [*Glyceria maxima* (Hartman) Holmb.], softstem bulrush [*Schoenoplectus tabernaemontani* (C. C. Gmel.) Palla], yellow flag (*Iris pseudacorus* L.), and cattail (*Typha* spp. L.) (Conley et al., 1991; Hunter et al., 2001). Although the performance of these aforementioned "wetland" plants in wastewater treatment has been well-documented, their widespread use has been tempered by concerns of invasiveness in certain ecosystems and high rates of biomass production and subsequent decomposition, which necessitate harvesting and removal.

Our study investigated a sustainable alternative to traditional wetland plants in constructed wetlands, specifically saleable horticultural plants with remediation potential. Similar to obligate wetland species, aquatic garden plants also thrive in waterlogged environments and offer the potential benefits of phytoremediation and economical value. In addition, they provide aesthetic value to subsurface flow treatment wetlands, which is important to nurseries and greenhouses located in highly populated urban areas (Fraser et al., 2004; Wood et al., 1999). Few studies have examined the survival of aquatic garden plants in subsurface

flow-constructed wetlands and their ability to recover nursery runoff rates of N and P (Arnold et al., 1999, 2003; Holt et al., 1999; B. K. Maynard, personal communication). Our objective was to evaluate commercially important species and cultivars of aquatic garden plants in a simple laboratory scale wetland system within the controlled environment of a greenhouse for their ability to grow and recover N and P.

Materials and Methods

Plant culture. Seven herbaceous emergent plants were chosen for their aesthetic properties, commercial importance, and ease of propagation (Table 1) and maintained in a greenhouse at Clemson University's Biosystems Research Complex (lat. 34°N, Clemson, SC) during 2003 to 2004. Bengal Tiger canna (*Canna × generalis* 'Bengal Tiger'), Yellow King Humbert canna (*C. × generalis* 'Yellow King Humbert'), imperial taro (*Colocasia esculenta* 'Illustris') and Full Eclipse Louisiana iris were propagated from rhizome divisions of donated stock plants (Carolina Nurseries, Moncks Corner, SC). Corms and offsets were removed from Chinese water chestnut [*Eleocharis dulcis* (Burman f.) Trin. ex Henschel] and green arrow arum (*Peltandra virginica*) stock plants, respectively (Charleston Aquatic Nursery, Johns Island, SC). Tissue-cultured plantlets of Singapore Pink pickerel-rush (*Pontederia cordata* 'Singapore Pink') were purchased from a commercial tissue culture laboratory (Agri-Starts II, Apopka, FL). Individual rooted plants were transplanted into 3601 cell packs (≈5

cm pots) containing a peat/vermiculite growing substrate (Fafard Germination Mix; Fafard, Anderson, SC) and maintained on the greenhouse bench in water-filled plastic-lined trays. Plants were watered and fertilized as needed.

A simple laboratory scale wetland system modeled after Fraser et al. (2004) and Wood et al. (1999) simulated a subsurface treatment wetland and was approximated as a batch system instead of a flow-through system. The wetland system was comprised of two polyethylene pots: a 16.5-cm diameter "azalea" pot (12.4-cm bottom diameter, 12.2-cm tall; Belden Plastics, St. Paul, MN) with bottom drainage holes, filled with pea gravel, and placed inside a 16.7-cm diameter aquatic pot with no drainage holes (13.2-cm bottom diameter, 16.5-cm tall; ITML, Brantford, Canada) so their rims were even (Fig. 1). The pea gravel had the following size distribution (% weight): less than 8 mm (33%); 8 to 15 mm (55%), and 15 to 20 mm (12%).

Determination of P-sorption by gravel. An equilibrium isotherm experiment was conducted to rule out the possibility of P-sorption by pea gravel. Approximately 31 g of gravel were placed into each of 36 50-mL acid-washed polyethylene centrifuge tubes. Aliquots (35 mL) of a 0.01 M KCl and Milli-Q water solution were spiked with ascending quantities of KH_2PO_4 to yield one of six P levels (0, 0.1, 1.0, 10, 100, and 1000 $\text{mg}\cdot\text{L}^{-1}$ P). The bottles were sealed with screw caps and continuously agitated in a rotary shaker table (Laboratory-Line Instruments, Melrose Park, IL) for 24 h at 22 °C. After settling, two aliquots (1.5 mL) of supernatant from each

Table 1. Species, family, cold hardiness, and description of the seven aquatic garden plants evaluated for their ability to recover runoff rates of nitrogen and phosphorus.^a

Species	Family	USDA cold hardiness zone	Description
<i>Canna × generalis</i> 'Bengal Tiger'	Cannaceae	7 to 10	Imported from India in 1963, this green and yellow variegated canna grows 1.2 to 1.8 m tall and bears bright orange flowers
<i>Canna × generalis</i> 'Yellow King Humbert'	Cannaceae	7 to 10	This sport of the red-flowered King Humbert canna bears yellow flowers dotted with orange and grows 1.2 to 1.5 m tall
<i>Colocasia esculenta</i> 'Illustris'	Araceae	7 to 10	Imperial taro has blackish purple leaves highlighted with green veins and grows 0.3 to 0.9 m tall
<i>Eleocharis dulcis</i>	Cyperaceae	9b to 11	Chinese water chestnut has been cultivated for centuries in China and southeast Asia; its bright green hollow shoots grow 0.9 m high, and edible nutlike tubers are borne at the ends of rhizomes
Iris, Louisiana Iris hybrid Full Eclipse	Iridaceae	6b to 10b	Introduced in 1978 Full Eclipse Louisiana iris produces very dark purple flowers and reaches a height of 0.9 to 1.2 m.
<i>Peltandra virginica</i>	Araceae	5 to 9b	Arrow or bog arum has glossy, dark green arrow-shaped leaves and produces a fleshy spike of green, pale yellow to white flowers and grows 0.6 to 0.9 m tall
<i>Pontederia cordata</i> 'Singapore Pink'	Pontederiaceae	6 to 11	This sport of pickerel-rush grows 0.3 to 0.6 m tall and produces pink flower spikes

^aCaillet et al., 2000; Speichert and Speichert, 2004.

Fig. 1. Sideview diagram of the laboratory scale wetland comprised of a pea gravel-filled, 16-cm diameter azalea pot inserted inside a 3-L aquatic pot.

sample were filtered through 0.2-mm polytetrafluoroethylene (PTFE) membrane filters, and the samples were analyzed for P using a Dionex AS50 ion chromatograph (IC) with AS50 autosampler (Dionex Corp., Sunnyvale, CA). The P amount removed from the solution by sorption to gravel was calculated by comparing the final aqueous P concentration with the initial aqueous P concentration. The data were then plotted using sorbed (dependent) and aqueous (independent) P concentrations. The appropriate isotherm relationship was determined from these plots and their correlation coefficients. Our data showed no definitive isotherm (Temkin, Freundlich, or Langmuir) relationship, which indicated no detectable adsorption of P by the pea gravel.

Two to 4 weeks before the initiation of an experiment, 40 to 50 liners of each species were removed from their containers, their roots washed free of substrate, weighed, and transplanted into gravel-filled azalea pots. After inserting the azalea pot into the aquatic pot; ≈ 1.350 L of a 10% modified Hoagland's solution (Hoagland and Arnon, 1950) was added to each pot until water appeared at the

gravel surface. During the acclimation period, plants were watered every 2 or 3 d to maintain the water level just below the gravel surface.

The average daily temperatures, relative humidity, and daily light integral (photosynthetically active radiation) over the course of the greenhouse study are listed in Table 2. During winter, a 16-h photoperiod was maintained with 1000-W metal halide lights.

Treatments. Five treatment levels of a modified Hoagland's solution ("solution 1" using nitrate-nitrogen) contained the following average concentrations of N and P ($\text{mg}\cdot\text{L}^{-1}$): 1) 0.39 N, 0.07 P; 2) 1.75 N, 0.18 P; 3) 10.44 N, 1.86 P; 4) 21.57 N, 3.63 P; and (5) 36.81 N, 6.77 P. Mean concentrations of the nutrient solutions, derived from random sampling and analyzing by inductively coupled plasma over the course of the study, encompassed the typical range of nutrients found in constructed wetland discharge, nursery runoff, and nursery irrigation water (Taylor et al., 2006). The initial pH of the nutrient solution was adjusted to 6.2 with 6 N H_2SO_4 .

Thirty acclimatized plants and six gravel-only pots were lifted from their aquatic containers, flushed with deionized water, and then returned to aquatic pots that had been emptied and rinsed with deionized water. On day 0, the appropriate treatment solution was batch-loaded into the pots with plants until it was visible at the gravel surface. Gravel-only pots received 10.44 and 1.86 $\text{mg}\cdot\text{L}^{-1}$ N and P, respectively. Thereafter, nutrient solution was added every 2 d to maintain the water level at the gravel surface.

Containers were arranged in a randomized complete block design with six replicates. The number of replicates was determined in a preliminary experiment with cultivar 'Yellow King Humbert'. Experiments were replicated twice for each species during the time periods listed in Table 2.

Data collection. Volumes of nutrient solution supplied to each container were

recorded during the 8-week experiment. After termination, water samples from the aquatic containers were filtered through 0.2-mm PTFE membrane filters into 1.5-mL IC vials and stored at 4 °C until anion analysis with a Dionex AS50 IC with AS50 autosampler to determine the percentage of recovered nutrient [(mg N or P supplied – mg nutrient remaining in solution) $\div$ mg N or P supplied] $\times 100$].

Aboveground portions of each plant were removed at the gravel surface and weighed. Belowground portions, which included roots that had grown through the drainage holes of the gravel-filled azalea pots, were placed over a screen and washed with tap water, rinsed with distilled water, and then weighed. Roots and shoots were dried at 80 °C, weighed, and ground in a Wiley mill (Swedesboro, NJ) to pass through a 40-mesh (0.425-mm) screen. N concentration was determined using 100 mg of tissue and assayed by an Elementar Vario Macro Nitrogen combustion analyzer (Mt. Laurel, NJ) with tissue analysis procedures described by Clemson University's Agricultural Service Laboratory (Anonymous, 2000). Phosphorus was assayed by wet acid digestion procedure using the nitric acid and hydrogen peroxide method (Anonymous, 2000; Mills and Jones, 1996). Phosphorus concentration was determined by inductively coupled plasma emission spectrophotometer (61E Thermo Jarrell Ash, Franklin, MA).

Because growth may dilute concentration, N and P contents were determined by multiplying plant part dry weight by nutrient concentration. Above- and belowground mineral contents were combined to provide whole plant N and P content.

Statistical analysis. Analysis of variance (ANOVA) was used to test for significant treatment (N and P concentrations), replication, and block effects. Because ANOVA indicated no rep and block effects but significant treatment effects, data were pooled. To determine

Table 2. Experiment dates, average daily temperature, relative humidity, and total photosynthetically active radiation for each species in two replicated experiments conducted in the Biosystems Research Complex greenhouses, Clemson University, Clemson, SC.

Species	Expt. 1			Expt. 2		
	Temperature (°C)	Relative humidity (%)	Daily light integral ($\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	Temperature (°C)	Relative humidity (%)	Daily light integral ($\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)
<i>Canna</i> $\times$ <i>generalis</i> 'Bengal Tiger'	23.3 $\pm$ 0.2	11 Feb. 2004 to 7 Apr. 2004 47.4 $\pm$ 1.5	16.1 $\pm$ 1.0	21.5 $\pm$ 0.2	7 Jan. 2004 to 2 Mar. 2004 48.4 $\pm$ 1.0	10.0 $\pm$ 0.8
<i>Canna</i> $\times$ <i>generalis</i> 'Yellow King Humbert'	26.7 $\pm$ 0.2	6 Aug. 2003 to 30 Sept. 2003 70.0 $\pm$ 0.8	21.0 $\pm$ 0.5	21.6 $\pm$ 0.2	5 Jan. 2004 to 2 Mar. 2004 48.8 $\pm$ 1.1	10.1 $\pm$ 0.8
<i>Colocasia esculenta</i> 'Illustris'	27.3 $\pm$ 0.07	24 June 2003 to 18 Aug. 2003 74.3 $\pm$ 0.3	22.1 $\pm$ 0.8	24.6 $\pm$ 0.2	8 Sept. 2003 to 3 Nov. 2003 61.5 $\pm$ 1.0	19.1 $\pm$ 0.9
<i>Eleocharis dulcis</i>	27.1 $\pm$ 0.1	24 July 2003 to 19 Sept. 2003 71.4 $\pm$ 0.7	21.6 $\pm$ 0.4	21.4 $\pm$ 0.2	9 Dec. 2003 to 3 Feb. 2004 49.7 $\pm$ 1.2	9.8 $\pm$ 0.7
Louisiana iris hybrid 'Full Eclipse'	24.5 $\pm$ 0.2	10 Sept. 2003 to 6 Nov. 2003 61.9 $\pm$ 1.1	16.1 $\pm$ 0.8	24.3 $\pm$ 0.2	18 Sept. 2003 to 14 Nov. 2003 61.0 $\pm$ 1.2	17.0 $\pm$ 0.9
<i>Peltandra virginica</i>	26.3 $\pm$ 0.2	13 Aug. 2003 to 7 Oct. 2003 67.0 $\pm$ 1.1	20.5 $\pm$ 0.5	21.4 $\pm$ 0.1	8 Dec. 2003 to 3 Feb. 2004 49.8 $\pm$ 1.1	9.9 $\pm$ 0.6
<i>Pontederia cordata</i> 'Singapore Pink'	23.5 $\pm$ 0.2	6 Oct. 2003 to 1 Dec. 03 58.9 $\pm$ 1.5	13.6 $\pm$ 0.8	23.8 $\pm$ 0.2	2 Oct. 2003 to 26 Nov. 2003 59.2 $\pm$ 1.4	14.4 $\pm$ 0.8

the nature of the treatment effect, regression analyses were performed for each species to describe changes in biomass and nutrient recovery relative to N or P supplied. Regression analysis showed a significant slope for biomass and nutrient use efficiency for each species. Therefore, slopes among species were compared using linear contrasts and *F* tests. Differences between shoot and root concentration and content were determined by Student's *t* tests. All calculations were performed with SAS (version 9.1 for Windows; SAS Institute, Cary, NC), and all tests used $P \leq 0.05$.

Results and Discussion

Biomass production. Growth rates for the seven species increased linearly and were highly correlated with increasing levels of nitrogen and phosphorus over the 8-week period (Fig. 2A–B), indicating that as plants increased in size and dry weight (DW), they assimilated correspondingly greater amounts of N and P. As a result of their higher evapotranspiration rates, cultivars ‘Bengal Tiger’ (*Canna* ‘BT’) and ‘Yellow King Humbert’ (*Canna* ‘YKH’) were supplied with greater amounts of N and P than the other species. Gravel-only pots receiving 10.44 and 1.86 mg·L⁻¹ N and P, respectively, were supplied with 62% to 86% less N and 52% to 86% less P than planted pots receiving the same level of N and P (data not presented).

Over the 8-week period, the rate of dry weight accumulation in the cannas was greater than the rest of the species (Fig. 2A–B). *Colocasia* ‘Illustris’ (*Colocasia*), *Pe. virginica* (*Peltandra*), and *Po. cordata* ‘Singapore Pink’ (*Pontederia*) produced biomass more rapidly than *E. dulcis* (*Eleocharis*) and *I. ‘Full Eclipse’* (*Iris*). Interestingly, *Peltandra* received the least amount of N and P over the 8-week period but exhibited a higher DW accumulation rate than *Eleocharis* and *Iris* (Fig. 2A–B). When supplied with the two lowest levels of N and P, the cannas exhibited more severe visual nutrient deficiency symptoms than the other five species, which included stunted growth and chlorotic older leaves.

Nitrogen and phosphorus recovery. Nitrogen and P recovery rates of the seven species were evaluated by comparing the amount of N or P supplied and recovered in whole plant tissues to a theoretical recovery rate in which the amount of N or P supplied equaled the amount of N or P recovered in the tissues. Nitrogen and P content of whole plant tissues for all seven species increased linearly with increasing concentrations of N and P and was highly related to the amount supplied to each species (Fig. 3A–B). *Canna* ‘BT’ and ‘YKH’ received the greatest N amounts; however, their N recovery rates were less than *Iris* and *Pontederia* (Fig. 3A). The N recovery rate of *Iris* was similar to the theoretical recovery rate of N (total N supplied = total N in tissues). *Eleocharis* and *Peltandra* had the lowest N recovery rates (Fig. 3A).

The cannas also received more P than the other five species over the 8-week period;

Fig. 2. The effect of (A) nitrogen and (B) phosphorus on whole plant dry weight of seven greenhouse-grown containerized aquatic garden plants over an 8-week period. Five concentrations of modified Hoagland’s solution (Table 3, footnote z) were initially batch-loaded and then supplied every 2 d to maintain the water level at the gravel surface. Vertical bars $\pm$ SE. Data points are the means of 12 plants. Slopes of the regression lines were compared using linear contrasts and *F* tests; species with different letters have significantly different slopes ($P \leq 0.05$).

however, the recovery rate of *Canna* ‘BT’ and ‘YKH’ were similar to *Iris*, *Peltandra*, and *Pontederia*, which were supplied with less P (Fig. 3B). *Colocasia* and *Eleocharis* exhibited the lowest P assimilation rate. None of the seven species assimilated P similar to the theoretical P recovery rate. The least amount of P supplied was similar to the P concentration in treated nursery runoff water, suggesting that nursery and greenhouse crops receive P in excess of their needs. Thus, fertilization rates for these species could be significantly reduced without affecting growth.

An analysis of the water that remained in the pots after 8 weeks revealed no significant differences in the concentration of remaining N and P. Less than 4% of the original amount of N and P supplied to the plants remained regardless of species and treatment level (data not shown). Of the original amount of N and P supplied to gravel-only pots, 35% to 48% of N and 18% to 37% of P remained (data not shown). These findings were consistent with other studies that showed an improvement in nutrient removal when plants were present (Huett et al., 2005; Hunter et al., 2001; Tanner et al., 1995).

Fig. 3. (A) Nitrogen and (B) phosphorus recovered in whole plant tissues of seven greenhouse-grown aquatic garden species over an 8-week period. Five concentrations of modified Hoagland's solution (Table 3, footnote z) were initially batch-loaded and then supplied every 2 d to maintain the water level at the gravel surface. Vertical bars $\pm$ SE. Data points are the means of 12 plants. The dashed line represents an ideal 100% recovery rate. Slopes of the regression lines were compared using linear contrasts and *F* tests; species with different letters have significantly different slopes ($P \leq 0.05$).

Depletion of P in the gravel-only pots could have resulted from assimilation by the thin film of algae present near the gravel surface and from microorganisms in biofilm (Costerton et al., 1995), whereas depletion may have occurred through denitrification processes. It is unlikely that P precipitation occurred in the gravel-only pots because the pH was not basic enough (mean pH of 7.1) to promote precipitation of calcium-phosphate complexes. Modeling with Visual MintEq. 2.52, a chemical equilibrium computer program that calculates the speciation, solubility, and equilibrium of solid and dissolved phases of minerals in aqueous systems, further con-

firmed that P precipitation was not a likely transformation pathway for P removal from the nutrient solution (Gustafsson, 2007).

Nitrogen and phosphorus concentration. Mineral concentrations are typically reported in wetland plant nutrient recovery research, although the contents or weights of nutrients reveal differences in nutrient accumulation by plants. As expected, the differences in nutrient allocation in the shoots and roots within species varied by both concentration and content. The N shoot concentration exceeded the amount in roots at every N level supplied for *Canna* 'YKH', *Colocasia*, and *Peltandra* (Table 3). *Pontederia* and *Iris*

shoots had greater N concentrations than roots at treatment levels exceeding 10.4 and 21.6 mg·L⁻¹ N, respectively.

Concentrations of P in *Canna* 'BT' and *Pontederia* were greatest in shoots at every P treatment level. *Pontederia* 'Singapore Pink' responded similarly to a natural community of *Po. cordata* from Lobo Reservoir, Sao Paulo, Brazil (Barbieri and Esteves, 1991). In an earlier study, Barbieri et al. (1984) had found that *Po. cordata* was capable of storing 10 times more P in its tissues than was present in the surrounding water. In contrast, there were no P differences between the *Peltandra* shoots and roots at any treatment level. No trends were observed with other species.

Mills and Jones (1996) reported an N concentration from "five mature leaves from new growth" of a "hybrid canna lily (*Ca. × generalis*)" twice as great as we measured in our cannas. Phosphorus concentration in their hybrid canna was the same as the highest P treatment level in our study. In a 4-month microcosm study in Florida, DeBusk et al. (1995) reported that water canna (*Ca. flaccida* Salisb.) and *Pe. virginica* receiving "enriched" (75.7 mg·L⁻¹ N, 29.2 mg·L⁻¹ P) and "unenriched" (9.7 mg·L⁻¹ N, 1.7 mg·L⁻¹ P) dairy wastewater accumulated concentrations of N and P that were similar to those accumulated by the hybrid cannas and *Pe. virginica* in our study. Both N and P concentrations for *Pe. virginica* were within the range of natural stands growing in a tidal freshwater marsh in Virginia (Chambers and Fourqurean, 1991).

The concentration of P in *Po. cordata* from the DeBusk et al. (1995) study was within the range we found for *Po. cordata* 'Singapore Pink'; however, their highest N tissue concentration (25.7 mg·L⁻¹ N) was less than our highest N concentration (30.9 mg·L⁻¹ N). In contrast, a pond community of *Po. cordata* in South Carolina had much lower N concentrations (Boyd, 1975), but P concentrations were comparable to that accumulated in our 1.86 mg·L⁻¹ P treatment levels. Nitrogen concentration of *Po. cordata* 'Singapore Pink' at our highest treatment level was comparable to that found for *Po. cordata* in a gravel-soil subsurface flow-constructed wetland treating restaurant and resort wastewater in Nairobi, Kenya (Nyakang'o and van Bruggen, 1999). The fourfold higher P concentration measured by Nyakang'o and van Bruggen was likely attributable to the greater P composition in their effluent.

Nitrogen and P concentrations for *Eleocharis dulcis* were lower than the concentrations for a natural stand of *E. quadrangulata* [(Michx.) Roem. & J. Schult.] in South Carolina (Boyd, 1975), but within the range of *E. acuta* R. Br., *E. philippinensis* Svenson, and *E. spachelata* R. Br. growing in a constructed wetland in Australia (Greenway and Woolley, 1999).

Nitrogen and phosphorus content. Total nitrogen content/plant [plant dry weight $\times$ tissue N concentration] was greater in canna roots than shoots at the lower N treatment levels. At greater concentrations, there was more N in shoots than roots. *Canna* 'YKH'

Table 3. Nitrogen (N) and phosphorus (P) concentration and content of shoots and roots of seven aquatic garden plants grown for 8 weeks in a laboratory scale wetland and receiving five treatment levels of N or P incorporated in a modified Hoagland's nutrient solution containing all other nutrients at levels to support normal plant growth.^z

Treatment level	Concn				Content			
	N		P		N		P	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
	(mg·g ⁻¹)				(mg)			
<i>Canna</i> ‘Bengal Tiger’								
1	11.74	11.05	3.03**	2.29	85.52	134.46**	22.39	27.66
2	11.12	10.41	2.54*	2.17	96.11	124.76	22.06	25.76
3	12.40**	9.41	2.45**	1.92	133.50	149.11	26.24	30.41
4	14.29**	9.43	2.48**	1.94	216.63	180.68	39.08	37.16
5	17.18**	10.46	3.06**	2.18	411.25**	243.62	75.01**	50.32
<i>Canna</i> × <i>generalis</i> ‘Yellow King Humbert’								
1	8.09**	6.78	1.82	1.73	34.15	63.67*	7.11	16.11**
2	8.31**	6.92	1.79	1.78	40.25	83.03*	8.29	20.95**
3	11.07**	7.23	1.90**	1.54	72.36	95.92	12.11	21.38*
4	13.48**	7.95	1.95**	1.69	142.69	131.06	20.46	28.92
5	16.03**	8.98	2.27*	2.01	315.62**	201.84	44.64	45.61
<i>Colocasia esculenta</i> ‘Illustris’								
1	9.89**	6.91	1.06	0.91	17.97	32.28**	1.95	4.29**
2	10.32**	7.51	1.12	0.94	21.57	33.00*	2.22	3.91**
3	11.02**	7.81	1.28*	1.08	44.39	45.80	5.24	6.25
4	12.00**	9.13	1.60**	1.28	80.70**	59.88	10.85**	8.43
5	15.39**	10.25	2.21**	1.71	224.38**	96.71	32.21**	16.56
<i>Eleocharis dulcis</i>								
1	6.68	6.58	1.93**	1.23	54.12	42.68	14.90*	8.29
2	6.61	6.73	1.82**	1.26	60.42	50.48	16.08	10.36
3	7.69	7.50	1.88**	1.38	84.91	65.02	20.77	13.19
4	8.73	8.63	2.03	1.75	101.24	76.76	24.09	17.18
5	12.93*	10.29	2.35	2.28	169.40**	83.08	31.34*	20.00
<i>Louisiana Iris</i> hybrid ‘Full Eclipse’								
1	9.13	9.30	1.38*	1.09	109.02**	9.31	16.50**	1.07
2	10.15	10.47	1.53	1.37	108.69**	11.56	16.82**	1.56
3	12.22	11.05	1.76	1.64	148.38**	17.31	21.63**	2.60
4	15.53**	11.76	2.22	1.97	193.50**	19.78	27.06**	3.25
5	19.81**	16.12	2.89**	2.32	286.91**	33.97	42.04**	4.89
<i>Peltandra virginica</i>								
1	11.46*	9.03	2.11	1.96	32.17	71.63**	5.60	14.970**
2	13.56**	8.98	2.23	2.13	40.15	86.02**	6.53	20.200**
3	14.94**	9.96	2.48	2.33	50.61	81.80	8.22	18.343**
4	16.97**	11.65	2.86	2.77	76.78	121.02**	12.45	28.752**
5	19.17**	12.59	2.94	2.89	126.27	138.52	19.39	32.491**
<i>Pontederia cordata</i> ‘Singapore Pink’								
1	9.93	9.28	1.43**	0.91	54.67**	29.43	7.88**	2.90
2	9.12	8.70	1.43**	0.90	47.70**	30.79	7.46**	3.20
3	10.98**	9.20	1.69**	0.95	78.50**	47.96	12.10**	4.98
4	13.48**	10.05	2.11**	1.07	124.78**	55.32	19.40**	6.00
5	18.13**	12.80	2.88**	1.52	284.85**	88.65	45.16**	10.51

^zTreatments were initially batch-loaded and then supplied every 2 d to maintain the water level at the gravel surface.

¹1 = 0.39 mg·L⁻¹ N/0.07 mg·L⁻¹ P; 2 = 1.75 mg·L⁻¹ N/0.18 mg·L⁻¹ P; 3 = 10.44 mg·L⁻¹ N/1.86 mg·L⁻¹ P; 4 = 21.57 mg·L⁻¹ N/3.63 mg·L⁻¹ P; 5 = 36.81 mg·L⁻¹ N/6.77 mg·L⁻¹ P; 1 mg·L⁻¹ = 1 ppm.

***Mean separation by *t* test comparing N and P in shoots and roots within species at each treatment level with significant differences at *P* ≤ 0.05 and *P* ≤ 0.01, respectively.

exhibited this change in sink strength with roots containing nearly 46% more N than shoots at the lowest N treatment level; however, shoots contained 36% more N than roots at the greatest N treatment level. Leaf sink strength may have been compromised by N deficiency as manifested by the chlorotic older leaves and stunted growth. *Canna* apparently was not adapted to low levels of nutrients in water, which is similar to *Phragmites australis* (Romero et al., 1999). *Colocasia* reacted similarly and contained 64% less N in the shoots at the lowest N treatment level, but it stored 57% and 70% more N in shoots than in roots at the two greatest N treatment levels, respectively.

Iris and *Pontederia* shoots had more N content than roots at every N treatment level. Greater than 90% of N was recovered in *Iris*

shoots, in contrast to 61% to 76% of N found in *Pontederia* shoots. Conversely, roots were the dominant sink for *Peltandra*, storing more than 50% of the N at every treatment level, which was similar to the response of *Phragmites australis* growing in a subsurface flow-constructed wetland in New South Wales, Australia (Huett et al., 2005).

Canna 'YKH' contained 64% to 72% more P in roots than in shoots at concentrations 1.86 mg·L⁻¹ P or less. There were no statistical differences at the two greatest P concentrations, although the trend indicated an increase in shoot P with increasing P levels. *Canna* 'BT' responded similarly in shoot and root P content with 40% more P in shoots than in roots at the greatest P treatment level.

Phosphorus contents of *Iris* and *Pontederia* at every treatment level were 90% or

greater and 70% or greater, respectively, in shoots. *Pontederia* was the only species that concentration and content followed identical trends. *Peltandra* had 63% or greater P in roots at every treatment level. Phosphorus content of *Colocasia* shoots exceeded roots only at the two greatest P treatment levels.

Direct comparisons of N and P recoveries with other studies are confounded as a result of different retention times, water depths, initial nutrient concentrations, plant densities, and harvesting regimens. However, our results support the sustainable approach of using aquatic garden plants in constructed wetlands to absorb N and P from wastewater versus using traditional obligate wetland plants, especially those with the potential for becoming invasive.

According to Tanner (1996), plants used in constructed wetlands should be tolerant of

waterlogged conditions, have rapid propagation rates, establish rapidly, and have a high pollutant removal capacity. All of the taxa in our study except for *Eleocharis* satisfied these requirements. The low N and P recovery rates of *Eleocharis*, along with its hollow stems, which are prone to breaking, negate its usefulness to remediate nursery and greenhouse runoff. All other species showed promise in remediation/production systems. For example, plants with highly efficient N and P recovery rates such as *Pontederia* and *Iris* can be placed at the discharge end of constructed wetlands. Cannas are best sited near the inflow end of constructed wetlands because they assimilate high N and P concentrations. Additionally, cannas are well suited for subsurface flow-constructed wetlands because of their ability to “process” high volumes of nutrient-rich water, which reduces the amount of effluent that has to be discarded. This, however, reduces the availability of recycled wetland-treated water for irrigation, which is an important water conservation practice.

Besides commercial floriculture and nursery production, these attractive species have the potential to be used in retention ponds and rain gardens to capture and filter runoff in commercial and residential landscapes and golf courses. Of growing international interest are “natural swimming pools” that rely on potted, gravel-grown aquatic plants to maintain water quality by absorbing nutrients and supporting microbial growth (Dunnett, 2005; Kingsbury, 2006).

Further work needs to be done to determine hydraulic loading rates and retention times and species-specific tolerance of pesticides to allow nursery and greenhouse producers with limited growing space to customize their remediation/production areas. Also, research is needed with pilot scale-constructed wetlands to determine the effects of various mono- and polycultural plant densities on nutrient recovery, propagation and production, and marketable plant quality.

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