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Nitrogen and phosphorus remediation of aquatic garden plants in laboratory-scale constructed wetlands.

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NITROGEN AND PHOSPHORUS REMEDIATION OF AQUATIC GARDEN PLANTS IN LABORATORY-SCALE CONSTRUCTED WETLANDS

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by
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ABSTRACT

This research investigated the potential of growing marketable aquatic garden plants that also remediate nursery and greenhouse runoff in a subsurface-flow constructed wetland. The cost of wastewater treatment is offset by the production of revenue-generating horticultural crops. Aquatic garden plants that offer the dual benefits of nutrient remediation and aesthetic value may also be used in bioretention basins, rain gardens, buffer zones, and filter strips.

Fifteen commercially available aquatic garden plants were grown for 8 weeks in a laboratory scale subsurface wetland in a greenhouse and received nitrogen (N) and phosphorus (P) from Hoagland's nutrient solution every two days for eight weeks. The N and P rates (0.39 to 36.81 $mg \cdot L^{-1}$ of N and 0.07 to 6.77 $mg \cdot L^{-1}$ 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. Among rhizomatous plants, highest N recovery rate were found in Louisiana Iris hybrid 'Full Eclipse', *Canna* 'Bengal Tiger', *Canna* 'Yellow King Humbert', *Colocasia esculenta* (L.) Schott 'Illustris', *Peltandra virginica* (L.) Schott, and *Pontederia cordata* L. 'Singapore Pink.' The P recovery rates were similar for the cannas, Louisiana Iris 'Full Eclipse,' *Peltandra virginica*, and *Pontederia cordata* 'Singapore Pink.' Among the fibrous-rooted aquatic garden plants, highest N and P recovery rates were exhibited by *Thalia geniculata* f. *rheumoides* Shuey and *Oenenathe javanica* (Blume) DC. 'Flamingo.' Floating plants with the highest N recovery rates

were exhibited by water hyacinth (*Eichhornia crassipes* [Mart.] Solms.) and water lettuce (*Pistia stratiotes* L.). Phosphorus recovery rates were similar for water hyacinth, water lettuce, and dwarf redstemmed parrotfeather (*Myriophyllum aquaticum* [Vell.] Verdc.).

To determine the effect of N:P ratio on P recovery, *Typha latifolia* and *Canna* 'Bengal Tiger' were grown in a greenhouse-based laboratory-scale subsurface constructed wetland system with a 4-day hydraulic retention time for 8 weeks. Plants were supplied with the following N:P ratios: 6:1, 3:1, 1:1, 1:3 and 1:6. Mean total P concentrations ranged from 6.9 $mg \cdot L^{-1}$ (6:1) to 252.2 $mg \cdot L^{-1}$ P (1:6); nitrate-nitrogen (NO_3-N) was maintained at a constant mean level of 42.4 $mg \cdot L^{-1}$. Measured endpoints at 20, 40, and 60 d included height, biomass, nutrient recovery/allocation, and nutrient use efficiency. *Canna* and *Typha* whole plant N:P concentration was linearly correlated with N:P ratio of treatments. For the 1:3 and 1:6 treatments, *Canna* assimilated 40.7 and 30.6% of supplied P compared to 9.7 and 6.2% for *Typha*. Although both species exhibited luxury consumption of P, *Typha latifolia* was nitrogen-limited at the 1:1, 1:3, and 1:6 N:P ratios. The high P shoot and root concentrations of *Canna* in the 42N:252P treatment--19.8 and 11.6 $mg \cdot g^{-1}$, respectively, were significantly higher than the 3.0 and 4.4 $mg \cdot g^{-1}$ cattail shoot and root P, respectively. These high shoot and root P concentrations for *Canna* 'Bengal Tiger' have not been previously reported.

In summary, results of this research showed the differential uptake of N and P by commercially available aquatic garden plants and the ability of some species to recover N and P at levels comparable to traditional constructed wetland plants. Also, the N:P ratio of wastewater influent affects P assimilation and appears to be species-specific in nature.

DEDICATION

I dedicate the work and effort contained within these pages to Susan Reynolds Polomski, my spouse and soul mate. Yes, dear, you do complete me.

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Finally, I offer heartfelt thanks to my wife, Susan, for her enduring love and support during this journey and without whom it would never have been possible.

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CHAPTER I

LITERATURE REVIEW

Introduction

Traditional production of containerized nursery and greenhouse crops in soilless media involves inputs of fertilizers, growth regulators, insecticides, and fungicides. Nursery producers in the southeastern U.S. use inert, porous materials such as pine bark and sand or peat- and pine bark (Yeager et al., 2005). Mixtures of sphagnum peat, polystyrene, vermiculite or perlite is used in the production of floriculture crops (Nelson, 2002). The limited cation exchange capacity and anion retention quality of soilless substrates may result in excessive leaching of nutrients and pesticides when production is not managed appropriately (Handreck and Black, 1999; Schoene et al., 2006). These potential contaminants may move offsite in runoff via irrigation or precipitation events and pollute ground and surface water. Nitrate-nitrogen (NO_3-N) and soluble reactive phosphate ($H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}) runoff from nursery and greenhouse operations may lead to excessive algal and aquatic plant growth in surface waters, resulting in accelerated eutrophication. Nitrate can move freely through soil and drains easily into streams and lakes, whereas NH_4^+ is more readily adsorbed by clay particles or organic matter in the soil (Horne and Goldman, 1994).

In a general assessment conducted by the U. S. Environmental Protection Agency (U. S. EPA), 44% of river and stream miles, 64% of lake, pond and reservoir acres, and 30% of bay and estuarine waters were reported to be impaired, primarily from eutrophication (U. S. EPA, 2004). Generally, freshwater systems are P-limited and more

prone to P inputs, while N often limits primary production in estuarine and marine environments (Carpenter et al., 1998). Also, high levels of nitrates in drinking water can cause methemoglobinemia in infants ("Blue Baby Syndrome") and gastrointestinal cancer in adults (McDonald and Kay, 1988).

The presence of nutrients from nonpoint sources and their impact in aquatic systems has resulted in increasing interest and scrutiny from the public, environmental groups, governmental agencies, and elected officials (Reinhardt et al. 2006). Specifically, P has been identified as the most critical nutrient impacting freshwater eutrophication, and agriculture identified as a major contributor (U. S. EPA, 1996; Sharpley et al., 1999). From an economic perspective, cultural eutrophication of U. S. freshwaters results in potential annual losses of $2.2 billion (Dodds et al., 2009). The authors concede that this value may be an underestimate of actual losses to recreational water usage, waterfront property, recovery costs of threatened and endangered species, and drinking water.

Since the enactment of the Clean Water Act (1972), the U. S. EPA has enforced provisions related to point-source pollution. In 1999, EPA began enforcing the nonpoint source pollution controls specified in section 303(d) of the Clean Water Act, which mandates that all states implement a Total Maximum Daily Load (TMDL) program for all watersheds and bodies of water (U.S. EPA, 2000a). A TMDL as defined in Section 303(d)(1)(C) of the Clean Water Act is the maximum amount of pollutant that a waterbody can receive from point and nonpoint sources and still maintain its designated use and value (e.g., drinking water, fish and wildlife habitat, recreation, etc.). Recently

TMDLs of nutrients in agricultural runoff were adopted by environmental regulatory agencies in every state (Yeager, 2006). Nutrient-loading criteria for natural waters will eventually be established in every state. This follows a trend where state governments have been passing more stringent laws and regulations assessing and regulating nonpoint sources of pollutants beyond the scope of the provisions of the Clean Water Act. 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). Maryland's Water Quality Improvement Act made it the first state to require N and P management plans for almost all sectors of agriculture (Lea-Cox and Ross, 2001) leading to voluntary or mandatory adoption of nutrient management plans by agricultural producers in other U. S. states.

The maximum contaminant level (MCL) for NO_3^- in drinking water is 10 $mg \cdot L^{-1}$ (National Academy of Sciences, 1977). No federal limits on P contamination in freshwater have been established due to variations in size, hydrology, and depth of rivers and lakes, and regional differences in P impacts. However, U. S. EPA recommends that total P not exceed 0.05 $mg \cdot L^{-1}$ in any streams discharging into lakes or reservoirs and 0.10 $mg \cdot L^{-1}$ in streams or other flowing waters that do not (U. S. EPA, 1986). Daniel et al. (1998) and Heathwaite and Dils (2000) suggest that the critical P concentration that promotes eutrophication is even lower—from 0.01 to 0.02 $mg \cdot L^{-1}$. Smil (2000) contends that the nutrient supply (loading rate) rather than P or N concentration in water may be the key anthropogenic factor in the cultural eutrophication process.

In greenhouse production fertigation runoff can contain 100 $mg \cdot L^{-1}$ NO_3-N (Wood et al., 1999). In nursery crop production, nursery runoff NO_3-N concentrations range from 0.1 to 135 $mg \cdot L^{-1}$ (Alexander, 1993; Taylor et al., 2006; Yeager et al., 1993) and P levels from 0.01 to 20 $mg \cdot L^{-1}$ P (Alexander, 1993; Headley et al., 2001; James, 1995; Taylor et al., 2006). These cited N and P runoff ranges could be higher or lower in other nursery and greenhouse crop production systems. In a recent study of runoff from 11 production nurseries in southern California, Mangiafioco et al. (2008) detected median NO_3-N concentrations of 40.3 and 15.6 $mg \cdot L^{-1}$ and median orthophosphate (PO_4-P) concentrations of 2.96 and 1.18 $mg \cdot L^{-1}$ from irrigation and precipitation events, respectively. Runoff nutrient concentrations vary in these studies due to differences in fertilizers, application rates and methods of fertilization, crops grown, greenhouse temperatures, available sunlight, and evaporation rates; nevertheless, these findings accentuate the need to manage and ameliorate discharge to comply with federal and state regulations.

Best Management Practices (BMPs) for containerized plant production (Yeager et al., 2005) have been used by U. S. growers to maximize production efficiency with minimal impact to the environment. BMPs stress optimal fertilization rates with controlled-release fertilizers (CRFs), reduced irrigation volume, and cyclic irrigation events. Paradoxically, the BMPs for container substrate electric conductivity levels with CRFs recommend concentrations of 15 to 25 $mg \cdot L^{-1}$ for NO_3-N and 5 to 10 $mg \cdot L^{-1}$ P (Yeager et al., 2005). These pour-through and suction lysimeter levels of container leachate exceed EPA-mandated MCLs for NO_3-N and P. To reduce N and P in effluent,

researchers have adopted a holistic approach that includes optimizing fertility rates and uptake efficiency, reducing irrigation water volume and timing watering applications to minimize leachate, amending soilless substrates to retain water and nutrients, reclaiming and recycling irrigation runoff in detention basins, and using constructed wetlands to mitigate runoff, which is the focus of this dissertation.

Constructed Wetlands for Nutrient Remediation

Constructed wetlands (CWs) are engineered systems designed and constructed to treat wastewater with vegetation, soils, and associated microbial populations involving the same biological and physicochemical processes that occur in natural wetlands (Vymazal, 2005; Scholz, 2006). Constructed wetlands have been used for decades mostly for the treatment of domestic or municipal sewage, which largely focused on reducing nutrients, suspended solids, heavy metals, and pathogens (Brown and Reed, 1994; Campbell and Ogden, 1999). Few CWS for wastewater treatment were operating in the U. S. before 1986 (Brown and Reed, 1994). Success in municipal and industrial point source discharges led to the widespread use of CWs to treat many other types of wastewater including industrial and agricultural wastewaters, landfill leachate, and stormwater runoff (Vymazal, 2005). Constructed wetlands are effective in treating total suspended solids, nitrogen, and phosphorus, as well as for reducing metals, organics, and pathogens (e.g., Scholz and Lee, 2005; Mungasavalli and Viraraghavan, 2006; Vymazal, 2007; Kadlec and Wallace, 2009).

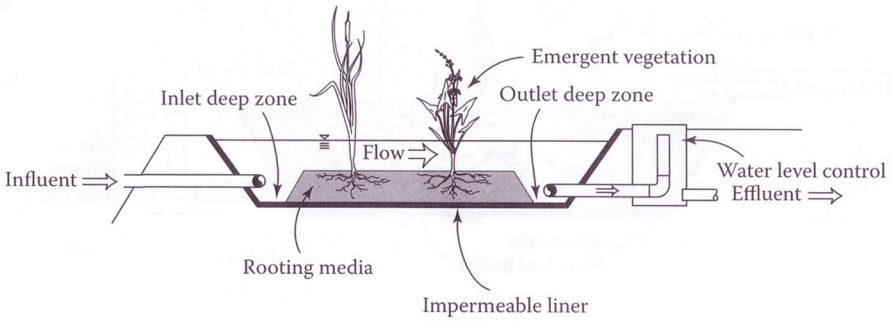
CWs are promoted as an inexpensive, low-cost technology to comply with increasingly stringent environmental regulations regarding the discharge of nonpoint

source pollutants in greenhouse and nursery production (Arnold et al., 1999; Berghage et al., 1999; Fernandez et al., 1999). This dissertation deals with the use of constructed wetlands for nutrient attenuation purposes.

Two types of constructed wetland systems exist: surface flow or free water surface (SF), and subsurface flow (SSF), which may be either horizontal or vertical flow CW systems (Kadlec et al., 2000). The types of macrophytes and water flow regimes differentiate these two CW systems. Free-floating, emergent, and submerged plants have been widely researched and used in CWs. Less performance data is available on CWs comprised of floating leaved and submerged plants. For the purposes of this dissertation, SSF CW wetlands are only being considered.

Surface-flow (SF) and SSF CWs are two commonly used wetland designs to treat agricultural wastewater (Berghage et al., 1999; Scholz and Lee, 2005; Taylor et al., 2006). A SF CW resembles a shallow (0.2-0.8 m) freshwater marsh and generally requires a large land area for wastewater treatment (Kadlec and Wallace, 2009) (Figure 1.1). To remediate nursery and greenhouse wastewater, surface area can be reduced with

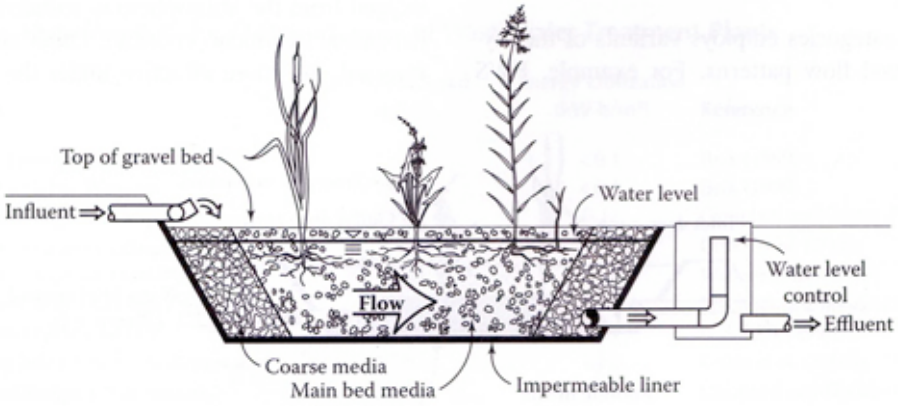
Figure 1.1. Basic construction of a free water surface wetland (adopted from Kadlec and Wallace, 2009).



a concomitant increase in depth (~1.25-1.5 m), which promotes anaerobic conditions that facilitate denitrification. The large land area required by typical SF constructed wetlands and the concomitant loss of production area has made them less attractive for greenhouse and nursery water treatment than SSF CWs (Berghage et al., 1999).

Alternatively, greenhouse and nursery operations constrained by limited production space and expensive land can use a SSF CW, which consists of a lined or impermeable basin filled with a 0.6 m deep coarse medium having high hydraulic conductivity, typically gravel, and wetland plants (Hunter et al., 2001; Kadlec and Wallace, 2009) (Figure 1.2). Wastewater flows horizontally or vertically below the

Figure 1.2. Basic construction of a horizontal subsurface flow constructed wetland (Adopted from Kadlec, 2007.)



surface of the media to prevent exposure to humans or wildlife. Subsurface flow CWs can be operated in continuous-flow or batch-load treatment modes with varying hydraulic residence times (Burgoon et al., 1995). Pretreated wastewater flows by gravity, horizontally or vertically, through the bed substrate where it contacts a mixture of facultative microbes living in association with the substrate and plant roots. The majority

of the saturated bed is anaerobic under most wastewater design loadings (Kadlec, 2009). In addition to agricultural effluent, horizontal flow SSF CWs are commonly used to treat other wastewater, such as municipal sewage, wastewaters from food processing, abattoir, pulp and paper production, textile industry, agriculture or landfill leachate (Vymazal et al., 1998; Vymazal and Kropfelova, 2008; Kadlec and Wallace, 2009).

Subsurface flow CWs are better for winter treatment compared to SF wetlands (Werker et al., 2002; Kadlec, 2009) and emit less total ammoniacal nitrogen (NH_3-N + NH_4^+-N) to the atmosphere; volatilization appears to play a more prominent role in the N budget of SF than SSF CWs (VanderZaag et al., 2008). However, the gravel substrate of SSF CWs is costly and has a finite treatment longevity because substrate clogging may occur after several years of operation (Moshiri, 1993; Kadlec and Wallace, 2009).

Transformation and Removal/Retention Mechanisms of Nitrogen and Phosphorus In Constructed Wetlands

Nitrogen

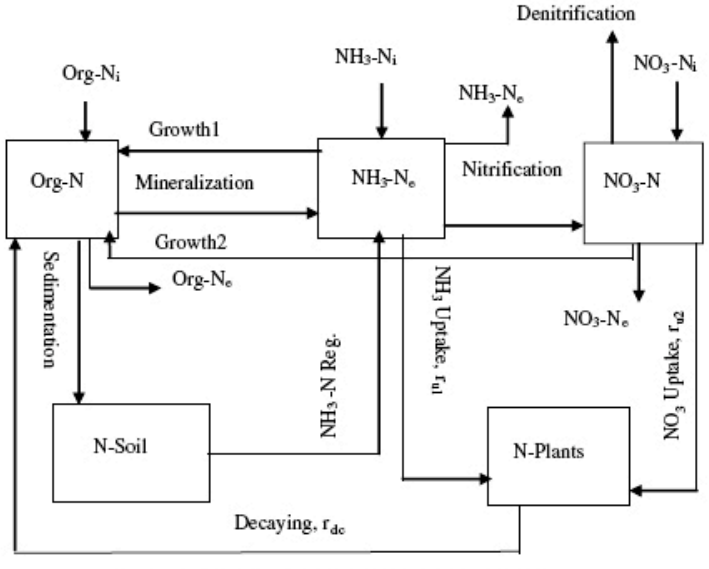
The transformation, retention, and removal of nitrogen (N) involves a complex set of processes and mechanisms that involve ammonification, nitrification–denitrification, adsorption, mineralization of organic nitrogen, ion exchange, fixation, biological uptake and assimilation, and volatilization (Scholz, 2006; Vymazal, 2007; Kadlec and Wallace, 2009) (Table 1.1 and Figure 1.2). The transformation processes, ammonification and nitrification, convert N to other forms and may lead to increases in the quantity of N in the system. Decomposition and mineralization processes in the wetlands are believed to convert a significant part of organic N, which is associated with particulate matter such as

organic wastewater solids and/or algae, to ammonia (Mayo and Mutamba, 2005). Nitrogen removal processes include ammonia volatilization, denitrification, plant uptake, ammonia adsorption, ANAMMOX and organic nitrogen burial.

Table 1.1. Major nitrogen transformation, retention, and removal processes in constructed wetlands. (Adopted from Vymazal, 2007.)

Ammonification (mineralization)	organic-N → ammonia-N	SF and SSF
Nitrification	ammonia-N → nitrite-N → nitrate-N	SF and SSF
Nitrate-ammonification	nitrate-N → ammonia-N	Horizontal SSF
Denitrification	nitrate-N → nitrite-N → gaseous N_2, N_2O	SF and SSF
N_2 Fixation	gaseous N_2 → ammonia-N (organic-N)	Mainly SF
Ammonia adsorption		SSF
Plant/microbial uptake (assimilation)	ammonia-, nitrite-, nitrate-N → organic-N	SF and SSF
Volatilization	ammonia-N (aq) → ammonia-N (g)	SF and SSF
Organic nitrogen burial		SF
ANAMMOX (anaerobic ammonium oxidation)	ammonia-N → gaseous N_2	SF and SSF

Figure 1.3. Nitrogen transformation and removal processes in a wetland. (Adopted from Mayo and Mutamba, 2005.)



Biological nitrification and denitrification are usually the most significant nitrogen removal mechanisms in SF and SSF CWs (Gersberg et al., 1984; Kadlec et al., 2005) and the dominant pathway of N removal from a wetland (Kadlec and Wallace, 2009). Other mechanisms such as plant uptake, substrate adsorption and ammonia volatilization are generally of less importance (Green, 1997; Kadlec and Wallace, 2009). Nitrification is a two-step acidifying that yields process that yields NO_3 with nitrite (NO_2) and protons as intermediary products. These chemical processes (Eq.[1], Eq.[2]) progress only when nitrifying bacteria (*Nitrosomonas*, *Nitrosoccus Nitrobacter, Nitrospira,* and *Nitrosovibrio*) are present to biologically oxidize NH_4^+ (Kadlec et al., 2000; Faulkner, 2004):

$$2NH_4^+ + 3O_2 \xrightarrow[\textit{Nitrosomonas}]{} 2NO_2^- + 2H_2O + 4H^+ \quad (1)$$

$$2NO_2^- + O_2 \xrightarrow[\textit{Nitrobacter}]{} 2NO_3^- \quad (2)$$

Nitrification is influenced by temperature, alkalinity and pH of the water, inorganic C source, moisture, microbial population, and concentrations of ammonium-N and dissolved oxygen (Vymazal, 2005). This process is generally more efficient in SF than SSF CWs (U.S. EPA, 2000b). Dissolved oxygen concentrations above 1.5 $mg \cdot L^{-1}$ are essential for nitrification to occur (Ye and Li, 2009).

Denitrification requires a carbon source, such as methanol or another equivalent carbon source and an anaerobic environment for denitrification to occur (Kadlec and Wallace, 2009). The denitrification process involves the transfer of electrons to nitrate

which results in the production of nitrogen gas (N_2), nitrous oxide (N_2O), or nitric oxide (NO):

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7NO_2 + 0.47N_2 + 1.68H_2O + HCO_3^-$$

The process is mediated by facultative bacteria that use nitrate as a final electron acceptor.

The N-removal efficiencies vary according to the type of CW (Table 1.2). Surface flow and horizontal flow SSF CWs provide anaerobic conditions that are more conducive to NO_3-N removal than vertical flow SSF CWs (Table 1.3). Alternatively, the highly oxygenated environment of vertical flow SSF CWs remove more NH_3-N than SF or horizontal flow SSF CWs. Thus, removal of both ammonium-N (NH_3-N) and NO_3-N species may best be accomplished in hybrid CW designs that provide anaerobic and aerobic conditions as exemplified by hybrid systems (e.g., Vymazal, 2005, 2007; Ye and Li, 2009) rather than single-stage CWs.

Biotic Assimilation of N

Ammonium and NO_3-N are the two forms of N assimilated by vegetation. However, biological assimilation includes microorganisms and algae. Plant species differ in their preferred forms of N; most plants, however, are capable of absorbing any form of soluble nitrogen, especially if acclimated to its presence (Atkin, 1996). The ammonium-nitrogen form is common in wetland habitats, especially where nitrification is restricted (Kadlec et al., 2000; Mitsch and Gosselink, 2007).

Table 1.2. Removal of total nitrogen (TN) in various types of constructed wetlands (mean values; adopted from Vymazal, 2007.)

CW Type	Unit	TN in	TN out	Efficiency	N
FFP[z]	$mg \cdot L^{-1}$	14.6	6.6	54.8	14
FWS[y]	$mg \cdot L^{-1}$	14.3	8.4	41.2	85
HSSF[x]	$mg \cdot L^{-1}$	46.6	26.9	42.3	137
VSSF[w]	$mg \cdot L^{-1}$	68.4	37.9	44.6	51

Loadings				Removed load	
FFP	$g \cdot m^{-2} \cdot yr^{-1}$	838	431	407	14
FWS	$g \cdot m^{-2} \cdot yr^{-1}$	466	219	247	85
HSSF	$g \cdot m^{-2} \cdot yr^{-1}$	644	394	250	113
VSSF	$g \cdot m^{-2} \cdot yr^{-1}$	1222	592	630	42

[z]FFP = free-floating plants (results from SE U.S., FWS[z] = free water surface systems (results from Australia, Canada, China, New Zealand, Poland, Sweden, The Netherlands, U.S.)
[x]HSSF = horizontal sub-surface flow (Australia, Austria, Brazil, Canada, Czech Republic, Denmark, Germany, India, Mexico, New Zealand, Poland, Slovenia, Sweden, UK, U.S.)
[w]VSSF = vertical sub-surface flow (Australia, Austria, China, Denmark, France, Germany, Ireland, Poland, Norway, The Netherlands, Turkey, UK).

Table 1.3. Removal of ammonia-N and nitrate-N in various types of constructed wetlands (mean values; adopted from Vymazal, 2007.)

CW Type	Inflow		Outflow	Efficiency (%)	N
	Concentration ($mg \cdot L^{-1}$)				
FWS	NH_4-N	12.9	5.8	55.1	64
	NO_3-N	5.6	2.2	60.7	57
HF	NH_4-N	38.9	20.1	48.3	151
	NO_3-N	4.4	2.9	38.5	79
VF	NH_4-N	55.0	8.7	84.2	80
	NO_3-N	0.7	24.4	NR[z]	62
Loading ($g\ N\ m^{-2} \cdot yr^{-1}$)					
FWS	NH_4-N	137	71	66	72
	NO_3-N	34	18	16	47
HF	NH_4-N	388	255	133	90
	NO_3-N	98	67	31	66
VF	NH_4-N	780	129	651	65
	NO_3-N	19.6	376	NR	46

[z]NR = not reported.

The potential rate of nutrient uptake by plant is limited by its net productivity (growth rate) and the concentration of nutrients in the plant tissue. Nutrient storage or standing stock is similarly dependent on plant tissue nutrient concentrations, and also on the ultimate potential for biomass accumulation: that is, the maximum standing crop. Therefore, desirable traits of a plant used for nutrient assimilation and storage would include rapid growth, high tissue nutrient content, and the capability to attain a high standing crop (Reddy and DeBusk, 1987).

In the literature there are many reviews on nitrogen concentrations in plant tissue as well as nitrogen standing stocks for plants found in natural stands and constructed wetlands (e.g., Reddy and DeBusk, 1987; Vymazal, 1995; Vymazal et al., 1999; Mitsch and Gosselink, 2007). Reddy and DeBusk (1987) reported the nitrogen standing stock for emergent species in the range of 14 to 156 g $N \cdot m^{-2}$, but the authors indicated that more than half of this amount could be stored belowground. Aboveground N standing stock values were reported in the range of 0.6 to 72 g $N \cdot m^{-2}$ (Johnston, 1991), 22 to 88 g $N \cdot m^{-2}$ (Vymazal, 1995), 2 to 64 g $N \cdot m^{-2}$ (Vymazal et al., 1999) or 2 to 29 g $N \cdot m^{-2}$ (Mitsch and Gosselink, 2007). Nitrogen standing stock may amount up to 250 g $N \cdot m^{-2}$ for water hyacinth (*Eichhornia crassipes*); multiple harvesting may yield N removal rates of 600 g $N \cdot m^{-2} \cdot yr^{-1}$ (Vymazal, 1995, 2001). Kadlec et al. (2005) reported that between 6 and 48% of nitrogen is retained by macrophytes planted in gravel-bed SSF CWs.

Various removal rates of total nitrogen by vegetation also exist in the literature. Brix (1994) reported N-uptake capacity of 200–2500 kg $N \cdot ka \cdot yr^{-1}$ of harvested emergent macrophytes, whereas the capacity of harvested submerged macrophytes is lower (<700

kg N·ka·yr^{-1}). Vymazal (2007) reported total N removal rates by vegetation between 40 and 55% with removed load ranging between 250 and 630 g N·m^{-2}·yr^{-1} depending on CWs type and inflow loading for various types of CWs.

Phosphorus Transformations, Retention, and Removal

Phosphorus in runoff is transported in either inorganic or organic forms and complexes that are soluble or insoluble (Table 1.4). The principal inorganic form of P is orthophosphate or soluble reactive phosphorus, the biologically available form of P. Orthophosphates are derived from inorganic fertilizer or released via decomposition of organic matter (e.g., vegetation and manures) and can be exported in dissolved, bioavailable forms—soluble reactive phosphorus. This labile dissolved P is removed via biological uptake (bacteria, phytoplankton, periphyton, and macrophytes) (Wetzel, 1990; Brix, 1997; Tanner, 1996), retained by amorphous and crystalline Fe or Al oxides, or by Ca (Khalid et al., 1978; Richardson, 1985), adsorbed to chemical compounds (iron, aluminum and calcium) in soils and sediments, and may be precipitated and/or co-precipitated in the water column (DeBusk et al., 2005). Free orthophosphate is the only form of P believed to be used directly by algae and macrophytes, which represents a major link between organic and inorganic phosphorus cycling in wetlands.

Dissolved organic compounds must first be converted into more labile forms before assimilation by aquatic biota (Newman and Robinson, 1999). Some P is readily released from dissolved organic compounds following exposure to UV radiation or to enzymes (Wetzel et al., 1995). In wetlands that successfully treat P to extremely low concentrations, recalcitrant dissolved organic P and particulate P compounds often

comprise the bulk of the outflow P (Dierberg et al., 2002).

In natural waters orthophosphate occurs in ionic equilibrium as

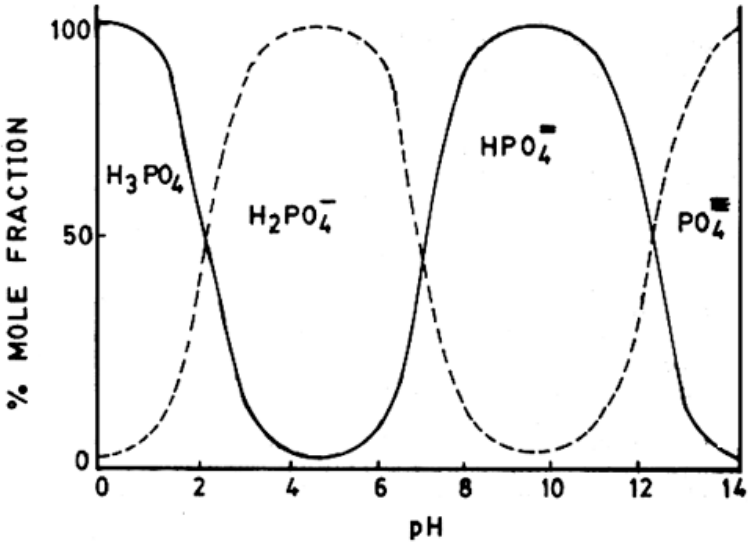
$H_3PO_4 \rightarrow H_2PO^{-1}_4 + H^+$ (pK = 2.2); $H_2PO_4 \rightarrow HPO^{-2}_4 + H^+$ (pK = 7.2); and $HPO_4 \rightarrow PO^{-3}_4 + H^+$ (pK = 12.3)

These forms are distributed in water at 25 ^oC as shown in Figure 1.4. Solubility of P in aquatic systems is regulated by temperature (Holdren and Armstrong, 1980), pH (Mayer and Kramer, 1986), redox potential (Moore and Reddy, 1994), interstitial soluble P level (Kamp-Nielson, 1974), and microbial activity (Gachter et al., 1998; Gachter and Meyer, 1993).

Dissolved P contained in inorganic fertilizer and released via decomposition of organic matter such as vegetation and manures, can be exported in dissolved, bioavailable forms—soluble reactive phosphorus. Dissolved P forms that enter a CW range from quite labile to extremely recalcitrant. Another group of inorganic phosphorus compounds are polyphosphates linearly condensed and cyclic. Dissolved organic P and insoluble forms of organic and inorganic P are generally not biologically available until they are transformed into soluble inorganic forms. Organic P forms can be generally grouped into 1) easily decomposable P (nucleic acids, phospholipids or sugar phosphates) and 2) slowly decomposable organic P (inositol phosphates or phytin) (Dunne and Reddy, 2005).

Phosphorus transformations, retention, and removal during wastewater treatment in CWs include the following processes: adsorption/desorption, precipitation/dissolution, plant and microbial uptake, fragmentation, leaching, mineralization, sedimentation (peat/soil accretion or deposition), and burial. Their pathways and various permanent and

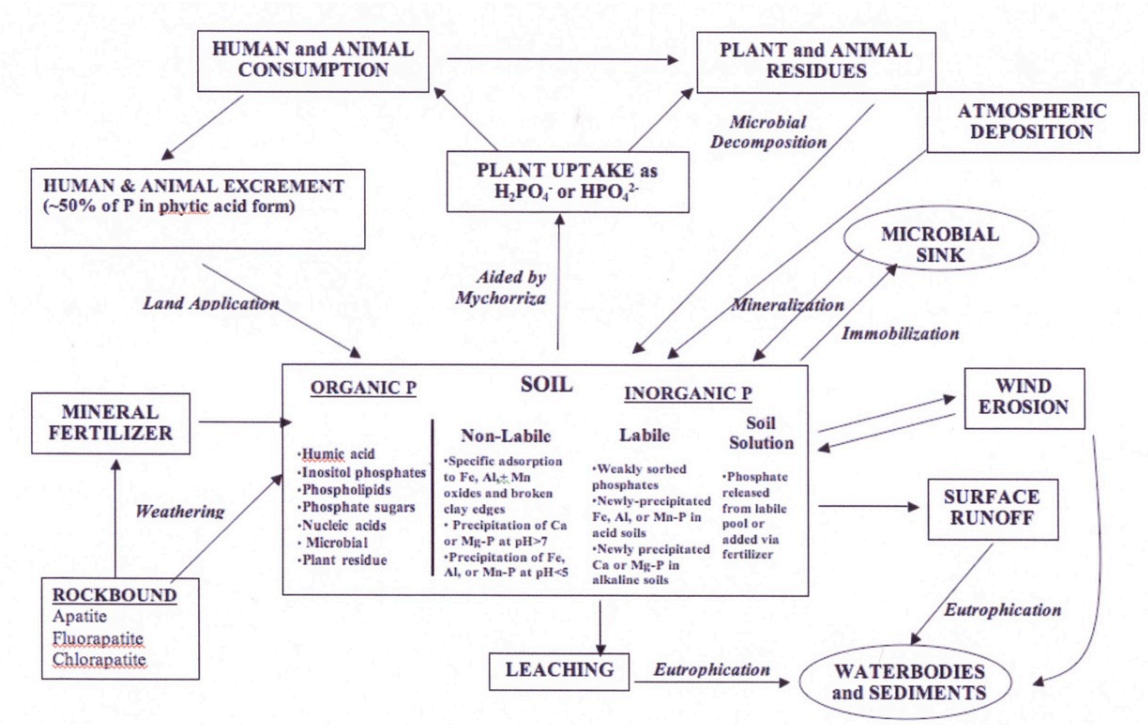
Figure 1.4. Distribution of phosphorus species expressed as a function of aqueous solution pH (adopted from Havlin et al., 2005)



temporary sources and sinks are illustrated in Figure 1.5. The major P removal processes are adsorption, precipitation, plant uptake (with subsequent harvest) and peat/soil accretion of new sediments and soils (Kadlec and Wallace, 2009). Horizontal-flow SSF systems have higher potential as the substrate is constantly flooded and there is not much fluctuation in redox potential in the bed. Vertical-flow SSF CWs may not be as effective because oxygenation of the bed caused by intermittent additions of wastewater may cause desorption and subsequent release of P. However, inert materials are commonly used in SSF CWS, such as washed gravel or crushed rock, and usually provide very low capacity for sorption and precipitation.

Precipitation involves the reaction of phosphate ions with metallic cations such as Fe, Al, Ca or Mg to form amorphous or poorly crystalline solids. These reactions typically occur at high concentrations of either phosphate or metalloid cations (Rhue and

Figure 1.5. Phosphorus biogeochemical cycle. (Adopted from Mitsch and Gosselink, 1986; Havlin et al, 2005; Kadlec and Wallace, 2009.)



Harris, 1999). Some important mineral precipitates in the wetland environment are in Table 1.4. In addition to direct chemical reaction, phosphorus can co-precipitate with other minerals, such as ferric oxyhydroxide and the carbonate minerals, such as calcium carbonate. The availability of Al, Fe, and Ca ions induces the precipitation of P into insoluble compounds depending on the pH and redox potential. At pH less than 5 the Al and Fe hydrous oxdides adsorb P. At pH greater than 7 Ca is the dominant adsorber. Low redox potential promotes solubilization of P (Sloey et al, 1978), which also transforms Al and Fe into amorphous forms that have a higher P sorption capacity (Patrick and Khalid, 1974).

Adsorption, precipitation, and biotic assimilation are saturable processes and

Table 1.4. Major types of soluble and insoluble phosphorus in the wetland environment (adopted from Reddy and D'Angelo, 1994; Devai et al., 1988; Devai and Delaune, 1995; Mitsch and Gosselink, 2007; Kadlec and Wallace, 2009).

Phosphorus	Soluble Forms	Insoluble Forms and Complexes
Inorganic	orthophosphates ($H_2PO_4^-$, HPO_4^{-2}, PO_4^{-3})	clay-phosphate complexes
	condensed phosphates: (pyro-,meta-, and poly- phosphates)	
	soluble reactive phosphorus: mainly PO_4-P with some condensed phosphates	
	ferric phosphate ($FeHPO_4^+$)	metal hydroxidephosphate
	calcium phosphate	apatite ($Ca_5(Cl,F)(PO4)_3$;
		hydroxylapatite ($Ca_5(OH)(PO4)_3$;
		variscite $Al(PO_4)\cdot 2H_2O$;
		strengite $Fe(PO_4)\cdot 2H_2O$;
		vivianite $Fe_3(PO_4)_2\cdot 8H_2O$; and wavellite $Al_3(OH_3)(PO_4)_2\cdot 5H_2O$
	phosphine (PH_3), gaseous water-soluble form of P	
Organic	dissolved organics, e.g., sugar phosphates, inositol, phosphates, phospholipids, and phosphoproteins	insoluble organic P bound in organic matter

therefore cannot contribute to long-term sustainable removal (Dunne and Reddy, 2005; Vymazal, 2007). Sedimentation is an important mechanism for removal of particulate inorganic and organic P and is considered to be the major long-term P storage in SF CWs where water overlies the sediment (Howard-Williams, 1985; Reddy et al., 1999; White et al., 2000). SSF CWs that use filtration substrate/particulate media and allow for contact between wastewater and filtration substrate can be designed to possess a large potential for P removal via adsorption and precipitation. Iron- and aluminum-rich materials, limestone media, and specially prepared clays have all been employed to enhance this

removal mechanism (Geohring et al., 1995; Jenssen and Krogstad, 2003; Vohla et al., 2007; Kadam et al., 2009).

Biotic Assimilation of Phosphorus

Both biotic and abiotic processes regulate phosphorus removal by wetlands. Biotic processes include uptake by vegetation and microorganisms, as well as mineralization of plant litter and soil organic phosphorus. Microbial uptake is considered in all treatment systems only as temporary storage of phosphorus with very short turnover rate. Phosphorus which is taken up by microbiota is released back to the water after the decay of the organisms.

The concentration of P in plant tissue varies among species and sites and during the season. Reddy and DeBusk (1987) reported P standing stock, i.e., the amount of phosphorus stored in aboveground biomass, for emergent species in the range of 1.4 to 37.5 g $P \cdot m^{-2}$ with more than 50% of this amount stored belowground. Aboveground P standing stock values were reported in the range of 0.1–6.8 g $P \cdot m^{-2}$ (Johnston, 1991), 0.1–11 g P m- 2 (Vymazal, 1995), 0.01–19 g $P \cdot m^{-2} \cdot yr^{-1}$ (Vymazal et al., 1999) or 3–15 g $P \cdot m^{-2}$ (Brix and Schierup, 1990). Phosphorus standing stock may amount up to 45 g $P \cdot m^{-2} \cdot yr^{-1}$ for *Eichhornia crassipes*. Due to its high productivity, the annual amount of P taken up by *E. crassipes* could be as high as 126 g $P \cdot m^{-2} yr^{-1}$ (Vymazal, 1995).

Annual P uptake rates by various macrophytes range between 0.77 and 40 g $P \cdot m^{-2} \cdot yr^{-1}$ for emergent species and between 10.5 and 126 g $P \cdot m^{-2} \cdot yr^{-1}$ for floating species (Vymazal, 2007). Phosphorus storage in aboveground biomass of emergent macrophytes is usually short-term, with a large amount of P being released as plant material senesces

and decomposes (Gaudet, 1977; Wetzel, 1990; Keuhn and Suberkropp, 1998; Keuhn et al., 1999). The rapid initial release of nutrients by leaching has been demonstrated in many wetland plants—up to 30% of nutrients are lost by leaching alone during the first few days of decomposition (Vymazal, 2007). However, dead roots decompose underground, therefore adding refractory compounds to sub-surface soils and leachates to the porewater in the root zone. Thus, the aboveground portions of macrophyte returns P to the water, while belowground portions decompose and add refractory P compounds to the soil returns P to the soil (Reddy et al., 1999). Removal of total phosphorus varied between 40 and 60% in all types of constructed wetlands with removed load ranging between 45 and 75 g $P \cdot m^{-2} \cdot yr^{-1}$ depending on CWs type and inflow loading (Vymazal, 2007).

Effect of N:P Ratios on N and P Assimilation

Nitrogen is the critical limiting element for growth of most plants (Smil, 1999; Socolow, 1999; Graham and Vance, 2000). Phosphorus is second only to N as the most limiting element for plant growth due to its unavailability (Bieleski, 1973; Vance et al., 2000). Both N and P are involved in plant metabolism and growth, and there are numerous points of interaction between N- and P-dependent processes. According to the Sprengel-Liebig Law of the Minimum (Epstein and Bloom, 2005), the most limiting nutrient controls plant growth. Therefore, if plants are deprived of an optimal P supply, then growth could be altered significantly. This has been demonstrated in experiments where plants assimilate nitrogen as NO^{3-} (Sutcliffe, 1962; Rufty et al., 1990). Alternatively, adding the limiting nutrient will increase nutrient uptake and growth.

This differential influence of N availability on uptake of other nutrients is reflected in the ratios of N to these other nutrients. By definition, N-ratios are a direct function of N uptake and an inverse function of the uptake of the other nutrient in the ratio (Gusewell, 2004b). The term "nutrient stoichiometry" is used to describe the nutrient ratios in plants (Mendez and Karlsson, 2005). Nutrient ratios long have been used to predict nutrient limitations. The Redfield ratio has been used as a measurement of nutrient limitations in natural waters (Redfield, 1958; Ketchum, 1969). It is based on the traditional view that phytoplankton and zooplankton need a relatively fixed atomic ratio of carbon to nitrogen to P of 106 to 16 to 1 (106 C: 16 N: 1 P). Removing C as a limiting nutrient, several primarily laboratory-based studies indicated that the average N:P ratio changes significantly relative to the conditions of the aquatic environment (Rast and Thornton, 1997; and references therein). The atomic ratios of N and P were changed to concentration ratios by Rast and Lee (1978) to allow for better measuring of N and P contents of waterbodies, yielding a 7.2 N:1 P ratio. Even though internal N:P ratios in algae differ from species to species (similar to plant species; Marschner, 1995) the Redfield ratio can be a useful tool for identifying limiting nutrient situations, if concentrations of the nutrients are static for at least a few days and light limitations are at a minimum (Hecky and Kilham, 1988).

In wetland ecology Koerselman and Meuleman (1996) proposed that critical foliar N:P values (i.e., threshold values) could be used to predict species and community-level N and/or P limitations. This proposal was based on 40 separate N and P nutrient addition experiments in European wetlands (bogs, fens, wet heathlands, dune slacks, and wet

grasslands). Their analysis demonstrated that N:P <14 was indicative of N limitation, N:P > 16 was indicative of P limitation, and N:P between 14 and 16 was indicative of N-P co-limitation. Although these critical N:P values were determined at the community level, the authors proposed that critical N:P values do not differ between individual plant species, citing that 11 of 40 studies were near monocultures. However, the universality of this ratio was disputed by Drenovsky and Richards (2004) who found species-specific critical N:P values for North American desert shrubs *Chrysothamnus nauseosus* ssp. *consimilis* and *Sarcobatus vermiculatus*, and that the N:P tool does not effectively predict desert shrub nutrient limitations.

In terrestrial ecology literature vegetation N:P ratios < 10 are indicative of N-limited environments (Gusewell, 2004b) and in certain agronomic crops N:P ratios < 5 are indicative of N-limted growing conditions (Duivenbooden et al., 1996). There is agreement that low N:P ratios indicate N limitation, but there is no consistent interpretation of high N:P ratios (Gusewell, 2004b). An important limitation of N:P ratios as predictors of nutrient limitation is they can only be applied to plants that are not limited by factors other than N or P (Koerselman and Meuleman (1996), which may be a problem in situations involving wastewater.

N:P ratio has been suggested as a tool for analyzing nutrient limitations and determining fertilizer requirements in agriculture and forestry (Gusewell, 2004a, 2004b; Koerselman and Meuleman, 1996; Tessier and Raynal, 2003). The Diagnosis and Recommendation Integrated System (DRIS) goes much further than the single nutrient ratio approach, in that it employs a minimum of three nutrient ratios per diagnosis, and

often as many as six or seven (Walworth and Sumner, 1987). DRIS was developed by Beaufils (1957, 1971) and has been shown to be capable of diagnosing the N, P, and K and in some cases Ca and MG requirements of a number of crops (Beverly, 1991).

In other words, the sufficiency status of an individual nutrient in plant tissue is diagnosed on the basis of its abundance relative to the abundances of at least two, and often as many as eight, other plant nutrients, thereby taking account of the state of nutrient balance within plant tissue. What is more, by simultaneously comparing the effects of different nutrients on crop yield, DRIS automatically ranks nutrient deficiencies or excesses in order of importance (Walworth and Sumner, 1987).

The N:P ratio of aquatic macrophytes is approximately 12:1 by weight (26.6 molar basis), although N:P ratios may differ between and among depending on nutrient availability, growth conditions, and the morphological stage of growth (Duarte, 1992). In many constructed wetland studies actual or simulated wastewater effluent is used without any regard for the N:P ratio in the effluent (e.g., Cizkova-Koncalova et al., 1996; Romero et al., 1999; Xie et al., 2004; and Kyambadde et al., 2005). Therefore, the proper N and P availability are of principal concern in the growth of wetland plants in constructed wetlands. However, the concentrations of nutrients, most importantly N and P, in the wastewater effluents and loading rate to the constructed wetlands vary depending on the quality of wastewater, type of wastewater treatment facilities, and the season. These changes to nutrient availability may influence plant growth responses and resource allocation in constructed wetlands (Adler et al., 2008; Zhang et al., 2008).

Numerous constructed wetland plant studies investigating effects of N and P on biomass and nutrient uptake of wetland plants typically vary N supply with the other nutrients remaining constant and nonlimiting or vary P supply with the other nutrients present at sufficient, nonlimiting levels. Alternatively, different amounts of the same nutrient solution with a N:P ratio less than 10 (mass:mass) or more than 70 were used (e.g., Elberse and Berendse, 1993; Cary and Weerts, 1984; Steinbachova-Vojtiskova et al., 2006). Plant growth was limited by N or P, and any differences in responses by species to treatments was primarily due to N or P supply. In other cases actual or simulated wastewater effluent was used without any regard for the N:P ratio in the effluent (e.g., Cizkova-Koncalova et al., 1996; Romero et al., 1999; Jing et al., 2001; Kyambadde et al., 2005). Fewer experiments compare plant growth responses and nutrient uptake to P supply or to combined variations in N and P supply and (e.g., Shaver and Melillo, 1984; Ulrich and Burton, 1985; Xie et al., 2004).

Gusewell (2005) proposed that responses to N and P may be specifically determined by the supply of N or P or by the supply of one relative to the other. This may be because the N:P supply ratio (supply of N relative to P) determines which of the two nutrients limits plant growth (Gusewell and Koerselman, 2002). Furthermore, functional differences may exist between N- and P-limited plants (Aerts and Chapin III, 2000). Some species appear to be most successful at high N:P supply ratios (e.g., *Molinia caerulea*; Kirkham, 2001; Tomassen et al., 2003), and others at low N:P supply ratios (e.g., *Typha glauca*; Woo and Zedler, 2002), suggesting that these species respond differently in terms of biomass production, morphology and/or physiology to the relative

supplies of N and P. Van der Heijden and Kuyper (2001) found that N:P supply ratio affects carbon gain, N and P-content, and root length of *Salix repens* and colonization of ectomycorrhizal fungi.

The few studies on the interactive effect of N and P on plant growth are inconsistent among wetland plant species. For example, Ulrich and Burton (1988) found that nitrate-nitrogen (NO_3-N) and P treatments and their interaction strongly affected plants growth and biomass of *Typha latifolia* L., *T. angustifolia* L., *Sparganium eurycarpum* Engelm., and *Phragmites austarlis* (Cav.) Trin. Ex. Steudel. However, Cary and Weerts (1984) and Romero et al. (1999) did not observe an interactive effect of N and P for either *Typha orientalis* Presl or *P. australis*. Romero (1999) found N supply affected growth of *P. australis* whereas P did not have any effect but imbalanced supply of N and P suppresses growth of P. *australis*.

Knowing how N:P supply ratios affect the growth and uptake of N and P may lead to improved plant selection in vegetated constructed wetlands and enable us to understand and predict how changes in relative supplies of N and P in wastewater affect N and P recovery.

Role of Plants in Constructed Wetlands

One of the many factors that control the efficiency of nutrient and bacterial removal in wetlands is vegetation type (Hammer, 1989), and macrophytes have both dominant and supporting roles in N and P recovery. Besides the direct assimilation of N and P from wastewater, the submerged portions of plants growing in SSF CWs provide a

large surface area for biofilms (Gumbricht, 1993, Chappell and Goulder, 1994), which are colonized by dense communities of photosynthetic algae and bacteria (Gumbricht, 1993; Chappell and Goulder, 1994; Brix, 1997). Microorganisms have the main role in the transformation and mineralization of nutrients and organic pollutants microorganisms (Stottmeister et al, 2003; Faulwetter et al., 2009). The plant species and types of substrate used in SSF CWs influence the dynamics and diversity of the rhizospheric bacterial community (Calheiros et al., 2009; Sleytr et al., 2009). In addition, roots support microbial activity through the exudation of carbohydrates, sugars, amino acids, enzymes, and many other compounds into the rhizosphere (Rovira, 1965, 1969; Ryan et al., 2001). The organic carbon exuded by the roots is the carbon source for denitrification by microbes (Rovira, 1965, Barber and Martin, 1976), which mediate most of the pollutant transformations occurring in wetlands (Kadlec et al., 2000). The metabolic investment in root exudates can be substantial: exudates of proteoid species can represent 5 to 25% of photosynthate production and can exceed 20% of total plant dry weight (Gardner et al., 1983; Dinkelaker et al., 1989; Johnson et al., 1996a, b).

Certain wetland plants oxidize the rhizosphere (Gersberg, 1986; Moorhead and Reddy, 1988; Burgoon et al., 1995), which also supports microbial growth and aids in the decomposition of organic matter. The release of oxygen by plant roots may increase the adsorption capacity of wetlands for P (Walhugala et al., 1987). Termed radial oxygen loss, this process has been well-described in numerous studies (Green and Etherington 1977; Mendelssohn and Postek, 1982; St-Cyr and Crowder, 1989; Roden and Wetzel, 1996). In addition, some species possess another more effective mechanism--through-

flow convection driven by pressure differences--to transport oxygen to belowground structures (Dacey, 1980, 1981). Some of the oxygen molecules support aerobic root respiration, while the rest diffuses toward the rhizosphere via the root surface. The oxygen-releasing root surface forms an oxidative layer that prevents the plant from absorbing phytotoxic reduced substrates such as Fe^{2+}, Mn^{2+}, and sulfide, which tend to accumulate in anoxic wetland sediments (Armstrong and Armstrong, 1988; Conlin and Crowder, 1988; Christensen et al., 1994).

Widely used plants in CWs include reed canarygrass (*Phalaris arundinacea* L.), common reed (*Phragmites australis* [Cav.] Trin. Ex Steud.), reed mannagrass (*Glyceria maxima* [Hartman] Holmb.), softstem bulrush (*Schoenoplectus tabernaemontani* [C. C. Gmel.] Palla), pickerel-rush (*Pontederia* spp.), *Scirpus* spp., *Juncus* spp., sedge (Cyperus spp.), yellow flag (Iris *pseudacorus*), and cattail (*Typha* spp. L.) (Wolverton, et al., 1983; Ansola et al., 1995; Cronk and Fennessy, 2001; Kadlec et al., 2000; Brisson and Chazarenc, 2009). These wetland plants have not been widely used commercially because of their potential invasiveness. Additionally in certain ecosystems and their high rates of biomass production, which necessitates periodic harvesting and removal to prevent the seasonal export of nutrients, particularly P via vegetative decomposition (Hunter et al., 2001). According to Tanner (1996) and Kvet et al. (1999), plants used in constructed wetlands should be tolerant of waterlogged conditions, have rapid propagation rates and easy to propagate sexually or asexually, establish rapidly, and have a high pollutant removal capacity and a long season of active vegetative growth.

Instead of traditional wetland plants, commercially available aquatic garden plants can be used in a production/remediation system that could generate revenue. Few studies have examined the ability of aquatic garden plants to thrive in SSF CWs and recover nursery runoff rates of nitrogen and phosphorus (Arnold et al., 1999; Holt et. al, 1999; Arnold et al., 2003; Belmont and Metcalfe, 2003; Zhang et al., 2007). The commercial value of aquatic garden plants offsets their production costs, which offers producers a sustainable, cost-effective and low maintenance remediation solution compared to conventional wastewater treatment technologies. Their usefulness could be expanded to other phytoremediation applications depending on the outcome of additional research assessing their ability to assimilate pesticides (e.g., Fernandez et al., 1999) and other anthropogenic pollutants (i.e., hydrocarbons, and metals) (e.g., Fritioff and Greger, 2003).

Plants Evaluated in These Studies

Apiaceae

Rainbow water parsley (*Oenenathe javanica* (Blume) D.C. 'Flamingo') is a low-growing Korean native with aromatic pink, white, and green leaves and an aroma of parsley. White umbels emerge in summer through fall and reaches a height of 15 cm.

Araceae

Imperial taro (*Colocasia esculenta* (L.) Schott var. *antiquorum* (Schott) Hubbard & Rehd. 'Illustris') has blackish-purple leaves highlighted with green veins and grows 0.3 to 0.9 m tall (Speichert and Speichert 2004).

Arrow- or bog arum (*Peltandra virginica* (L.) Schott.) has glossy, dark green arrow-shaped leaves that grows to a height of 0.6 to 0.9 m (Dalton and Novelo R., 1983; (eFloras.org, 2009). A green, tubular pointed spathe nearly conceals the clublike spadix of pale yellow to white flowers (Dalton and Novelo R., 1983; McIninch and Garbisch, 2003). The basal portion of the spadix contains pistillate flowers while the upper portion contains staminate flowers. Eventually green berries are produced in the fall.

Water lettuce (*Pistia stratiotes* L.) is a free-floating, stoloniferous aquatic that produces a rosette of light to lime-green velvety leaves; it can reach a mature height of 30.5 cm (Speichert and Speichert, 2004). It reproduces by offsets that grow from the base of the mature plant (Slocum, 1990). It is used to shade the surface of aquatic gardens to reduce algal problems.

Cannanaceae

Bengal Tiger canna (*Canna* 'Bengal Tiger'; syn. 'Pretoria') is a green and yellow-variegated canna that grows 1.2 to 1.8 m tall and produces panicles of orange and red-orange flowers (Ogden, 2007). It is adapted to terrestrial and water-inundated conditions.

Yellow King Humbert canna (*Canna* 'Yellow King Humbert) is a sport of the red-flowered 'King Humbert' canna (syn. 'Roi Humbert'; Ogden, 2007). It is a large-leaved herbaceous rhizomatous perennial cultivated for its broad, bananalike leaves and flowers that range in color from red, orange, or yellow or any combination of those colors. Often the flowers may be dotted or streaked with crimson. It can reach a height of 1.2 to 1.5 m in terrestrial landscapes and can also be grown in shallow aquatic environments (Speichert and Speichert, 2004; Ogden, 2007).

Cyperaceae

Chinese water chestnut (*Eleocharis dulcis* (Burman f.) Trin. ex Henschel) has been cultivated for centuries in China and southeast Asia. Its bright green hollow cylindrical shoots grow 0.3-0.1 m high, and edible nutlike tubers are borne at the ends of rhizomes (Slocum et al., 1996).

Starrush whitetop (*Rhyncospora colorata* (L.) H. Pfeiffer) is a free-flowering, rhizomatous North American native that ranges from SE VA south to south FL and west thru southern Texas and continues south through Mexico and Costa Rica (McMillan, 2007). It grows 30-61 cm tall and produces white starlike inflorescence comprised of 5-50 spikelets. It can be found in moist, saturated, or seasonally inundated environments.

Haloragaceae

Myriophyllum aquaticum is a perennial aquatic plant classified as a creeping emergent roots that freely in floating mats or anchored in substrate where it reproduces primarily by stem fragmentation (Sytsma, 1989). Dwarf redstemmed parrotfeather (*Myriophyllum aquaticum* [Vell.] Verdc.) is a compact selection with bright red prostrate or ascending stems bearing whorls of gray-green feathery leaves (Speichert and Speichert, 2004). Sexual reproduction also occurs by way of axillary, unisexual and perfect flowers present on the same plant. This species is considered a noxious weed in some areas due to its aggressive growth and reproduction; however, it is sold as an aquatic garden plant for its ability to oxygenate. (Speichert and Speichert, 2004).

Iridaceae

'Full Eclipse' Louisiana iris *Iris* x *louisiana 'Full Eclipse'* was introduced in 1978 by Ben Hager. This late midseason iris produces very dark purple flowers and reaches a height of 0.9 to 1.2 m. (Caillet et al., 2000). 'Full Eclipse' received the American Iris Society Award of Merit in 1985.

Pontederiaceae

Water hyacinth (*Eichhornia crassipes* [Mart.] Solms.) is a free-floating plant comprised of a rosette of petiolate leaves, an attractive lavender-blue inflorescence, and extensive submerged roots (Gopal, 1987; Speichert and Speichert, 2004). Although water hyacinth is used in aquatic gardens, it is considered one of the world's worst weeds (Holm et al, 1997). In natural systems water hyacinth propagates rapidly forming dense mats that spread out across the water surface, blocking traffic, destroying natural landscapes, affecting water quality, decreasing biodiversity, providing conditions for mosquitoes to breed, and retarding agricultural development (Holm et al. 1997; Mehra et al. 1999).).

Singapore Pink pickerel-rush is a sport of the native North American pickerel-rush (*Pontederia cordata* L. 'Singapore Pink') (Speichert and Speichert, 2004). This rooted emergent hydrophyte produces short, thick prostrate rhizomes and has long-petioled, parallel-veined leaves (Speichert and Speichert, 2004). Singapore Pink grows 0.3 to 0.6 m tall and produces pink flower spikes (Speichert and Speichert, 2004).

Marantaceae

Red-stemmed alligator flag (*Thalia geniculata* f. *rheumoides* Shuey) has reddish-purple petiole, sheath, and pulvinus and bears long arching flower spikes of silvery-purple flowers; grows 0.6 to 3 m tall and 0.6-1.8 m wide. It is widely distributed in parts of the Americas and W. Africa (eFloras.org, 2009).

Typhaceae

Common or broadleaved cattail (*Typha latiifolia* L.) is an emergent hydrophytic plant that grows up to 1.5-3 m in height in marshes and shallow water throughout the world. It tolerates a wide range of soil and water conditions--fresh to slightly brackish water; and is often among the first species to invade areas impacted by human activities (eFloras.org, 2009; USDA, 2009). Pale- to grayish-green leaves are distichously arranged, erect, essentially flat, and pale- to grayish-green in color (Correll and Correll, 1975; eFloras.org, 2009). Inflorescense is a dense, cylindrical spike comprised of two portions: upper contains staminate flowers and lower contains pistillate flowers. The pistillate flowers persist giving rise to dark brown showy fruit up to 7 inches long and 2 inches wide. Small single-seeded fruits are up to 20,000 to 70,000 per inflorescense. Plants spread by creeping rhizomes and seed dispersion. Cattails spread by creeping rhizomes and seed dispersion."

Miniature cattail (*Typha minima* Hoppe) is native to parts of the Middle East and central Asia. It reaches a garden height of 30-46 cm; brown marble-sized catkins rise above its 3-6 mm wide blue-green leaves (Speichert and Speichert. 2004).

Verbenaceae

Lanceleaf frogfruit (*Phyla lanceolata* [Michx.] Greene) is a creeping North American native that grows 5-10 cm high, tolerates light foot traffic, and produces tiny white flowers that fade to yellow and then pink (Speichert and Speichert, 2004). Foliage turn crimson-purple in the fall.

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CHAPTER II

NUTRIENT RECOVERY BY SEVEN AQUATIC GARDEN PLANTS IN A LABORATORY-SCALE SUBSURFACE CONSTRUCTED WETLAND

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 nitrogen (N) and phosphorus (P) from Hoagland's nutrient solution every two days for eight weeks. These rates (0.39 to 36.81 $mg \cdot L^{-1}$ of N and 0.07 to 6.77 $mg \cdot L^{-1}$ 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* 'Bengal Tiger', *Canna* 'Yellow King Humbert', *Colocasia esculenta* (L.) Schott 'Illustris', 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,'

Peltandra virginica (L.) Schott, 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 ground water. Excess nutrients, notably nitrate-nitrogen (NO_3-N) and soluble reactive phosphorus (PO_4^{3-}, $H_2PO_4^-$, $H_2PO_4^{2-}$, and H_3PO^{4-}), 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 NO_3-N contaminant levels in any discharged water at 10 $mg \cdot L^{-1}$ (U. S. EPA, 1986). Federal limits on 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 $mg \cdot L^{-1}$ and 0.1 $mg \cdot L^{-1}$, respectively (U. S. EPA, 1986). Greenhouse wastewater typically contains 100 $mg \cdot L^{-1}$ NO_3-N (Wood et al., 1999), while nursery runoff levels of NO_3-N range from 0.1 to 135 $mg \cdot L^{-1}$ (Alexander, 1993; Yeager et al, 1993; Taylor et al., 2006). A range of 0.01 to 20 $mg \cdot L^{-1}$ 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 twelve 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 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 N and P occurs via 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 mannagrass (*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 necessitates 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 economic 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 (Wood et al., 1999; Fraser et al., 2004). Few studies have examined the survival of aquatic garden plants in subsurface-flow constructed wetlands and their ability to recover nursery runoff rates of nitrogen and phosphorus (Arnold et al., 1999; Holt et al., 1999; Arnold, et al., 2003; 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 nitrogen and phosphorus.

Materials and Methods

Plant Culture

Seven herbaceous emergent plants were chosen for their aesthetic properties, commercial importance, and ease-of-propagation (Table 2.1) and maintained in a greenhouse at Clemson University's Biosystems Research Complex (lat. 34º N, Clemson, SC, USA) during 2003-2004. Bengal Tiger canna (*Canna* 'Bengal Tiger'), Yellow King Humbert canna (*C.* 'Yellow King Humbert'), imperial taro (*Colocasia esculenta* 'Illustris', and Full Eclipse Louisiana iris were propagated from rhizome divisions of donated stock plants (Carolina Nurseries, Inc., Moncks Corner, SC). Corms and offsets were removed from Chinese waterchestnut (*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 lab (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 Inc., 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

Table 2.1. Species, family, cold hardiness, and description of the seven aquatic garden plants evaluated for their ability to recover runoff rates of N and P (Caillet et al., 2000; Speichert and Speichert, 2004).

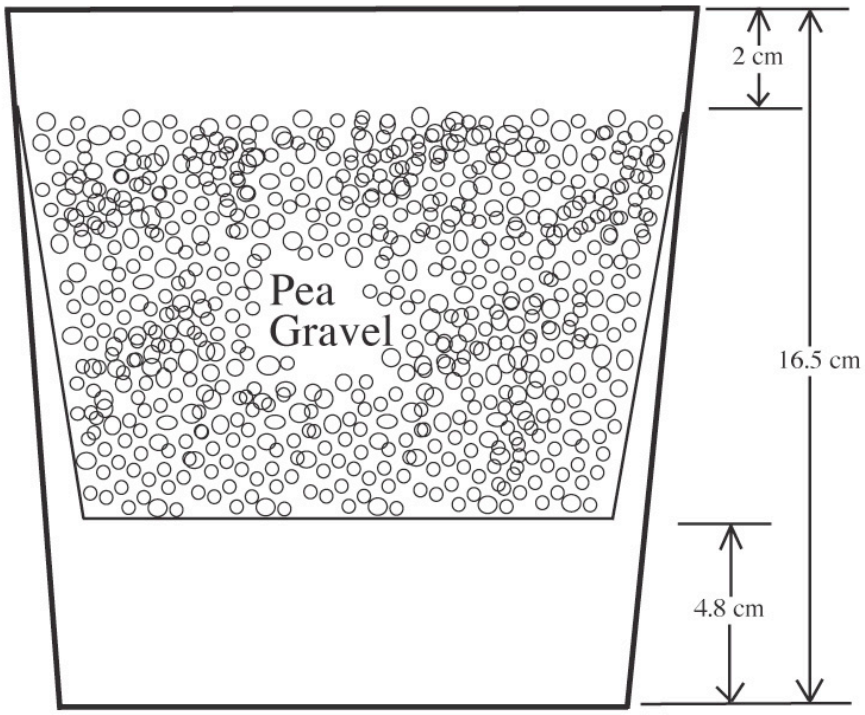
Species	Family	USDA cold hardiness zone	Description
Canna 'Bengal Tiger'	Cannaceae	7 to 10	Imported from India in 1963, this green and yellow-variegated canna grows 1.2 to 1.8 m tall and bears bright orange flowers.
Canna 'Yellow King Humbert'	Cannaceae	7 to 10	This sport of the red-flowered King Humbert canna bears yellow flowers dotted with orange and grows 1.2 to 1.5 m tall.
Colocasia esculenta 'Illustris'	Araceae	7 to 10	Imperial taro has blackish-purple leaves highlighted with green veins and grows 0.3 to 0.9 m tall.
Eleocharis dulcis	Cyperaceae	9b to 11	Chinese waterchestnut 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.
Peltandra virginica	Araceae	5a 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.
Pontederia cordata 'Singapore Pink'	Pontederiaceae	6a to 11	This sport of pickerel-rush grows 0.3 to 0.6 m tall and produces pink flower spikes.

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 (Figure 2.1). The pea gravel had the following size distribution (% weight): <8 mm (33%); 8-15 mm (55%), and 15-20 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 6 P levels (0, 0.1, 1.0, 10, 100, and 1,000 $mg \cdot L^{-1}$ P). The bottles were sealed with screw caps and continuously agitated in a rotary shaker table (Lab-Line Instruments, Melrose Park, Ill.) for 24 h at 22 ºC. After settling, two aliquots (1.5 mL) of supernatant from each sample were filtered through 0.2 mm polytetrafluoroethylene (PTFE) membrane filters, and the samples were analyzed for phosphorus using a Dionex AS50 IC with AS50 autosampler (Dionex Corp., Sunnyvale, CA.). The P amount removed from 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 (Figure 2.1).

Figure 2.1. 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.



Two to four 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, about 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 two or three days to maintain the water level just below the gravel surface.

The average daily temperatures, relative humidity, and daily light integral (*PAR*) over the course of the greenhouse study are listed in Table 2.2. During winter a 16-h photoperiod was maintained with 1000 W metal halide lights.

Table 2.2. Experiment dates, average daily temperature, relative humidity, and total *PAR* for each species in two replicated experiments conducted in the Biosystems Research Complex greenhouses, Clemson University, Clemson, SC.

	Experiment 1			Experiment 2		
Species	Temperature (°C)	Relative Humidity (%)	Daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)	Temperature (°C)	Relative Humidity (%)	Daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)
	11-Feb-04 to 7-Apr-04			7-Jan-04 to 2-Mar-04		
Canna 'Bengal Tiger'	23.3 ± 0.2	47.4 ± 1.5	16.1 ± 1.0	21.5 ± 0.2	48.4 ± 1.0	10 ± 0.8
	6-Aug-03 to 30-Sep-03			5-Jan-04 to 2-Mar-04		
Canna 'Yellow King Humbert'	26.7 ± 0.2	70.0 ± 0.8	21.0 ± 0.5	21.6 ± 0.2	48.8 ± 1.1	10.1 ± 0.8
	24-Jun-03 to 18-Aug-03			8-Sep-03 to 3-Nov-03		
Colocasia esculenta 'Illustris'	27.3 ± 0.07	74.3 ± 0.3	22.1 ± 0.8	24.6 ± 0.2	61.5 ± 1.0	19.1 ± 0.9
	24-Jul-03 to 19-Sep-03			9-Dec-03 to 3-Feb-04		
Eleocharis dulcis	27.1 ± 0.1	71.4 ± 0.7	21.6 ± 0.4	21.4 ± 0.2	49.7 ± 1.2	9.8 ± 0.7
	10-Sep-03 to 6-Nov-03			18-Sep-03 to 14-Nov-03		
Louisiana iris hybrid 'Full Eclipse'	24.5 ± 0.2	61.9 ± 1.1	18.5 ± 1.0	24.3 ± 0.2	61.0 ± 1.2	17.0 ± 0.9
	13-Aug-03 to 7-Oct-03			8-Dec-03 to 3-Feb-04		
Peltandra virginica	26.3 ± 0.2	67.0 ± 1.1	20.5 ± 0.5	21.4 ± 0.1	49.8 ± 1.1	9.9 ± 0.6
	6-Oct-03 to 1-Dec-03			2-Oct-03 to 26-Nov-03		
Pontederia cordata 'Singapore Pink'	23.5 ± 0.2	58.9 ± 1.5	13.6 ± 0.8	23.8 ± 0.2	59.2 ± 1.4	14.4 ± 0.8

Treatments

Five treatment levels of a modified Hoagland's solution ("Solution 1" using nitrate-nitrogen) contained the following average concentrations ($mg \cdot L^{-1}$) of N and P: (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 ICP 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 five 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 $mg \cdot L^{-1}$ N and P, respectively. Thereafter, nutrient solution was added every two days to maintain the water level at the gravel surface.

Containers were arranged in a randomized complete block design with 6 replicates. The number of replicates was determined in a preliminary experiment with *Ca.* 'Yellow King Humbert.' Experiments were replicated twice for each species during the time periods listed in Table 2.2.

Data Collection

Volumes of nutrient solution supplied to each container were recorded during the eight-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 4ºC 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 ÷ mg N or P supplied) x 100].

Above-ground portions of each plant were removed at the gravel surface and weighed. Below-ground 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 tapwater, rinsed with distilled water, and then weighed. Roots and shoots were dried at 80ºC, weighed, and ground in a Wiley mill 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 (Mills and Jones, 1996; Anonymous, 2000). Phosphorus concentration was determined by inductively coupled plasma emission spectrophotometer (61E Thermo Jarrell Ash, Franklin, MA).

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

Statistical Analysis

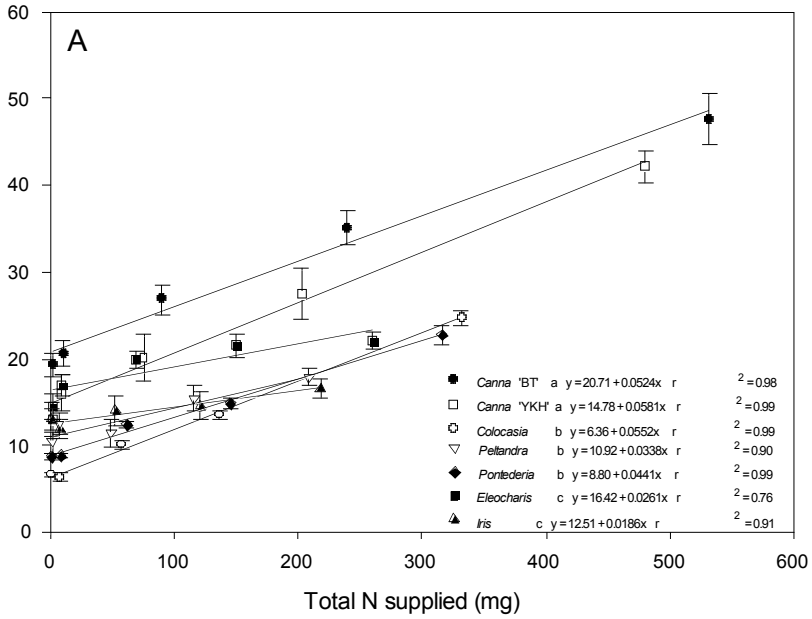
Analysis of variance (ANOVA) was used to test for significant treatment (N and P concentrations), rep, and block effects. Since ANOVA indicated no rep and block effects but significant treatment effects, data were pooled. To determine the nature of the treatment effect, regression analyses were performed 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 $P \leq 0.05$.

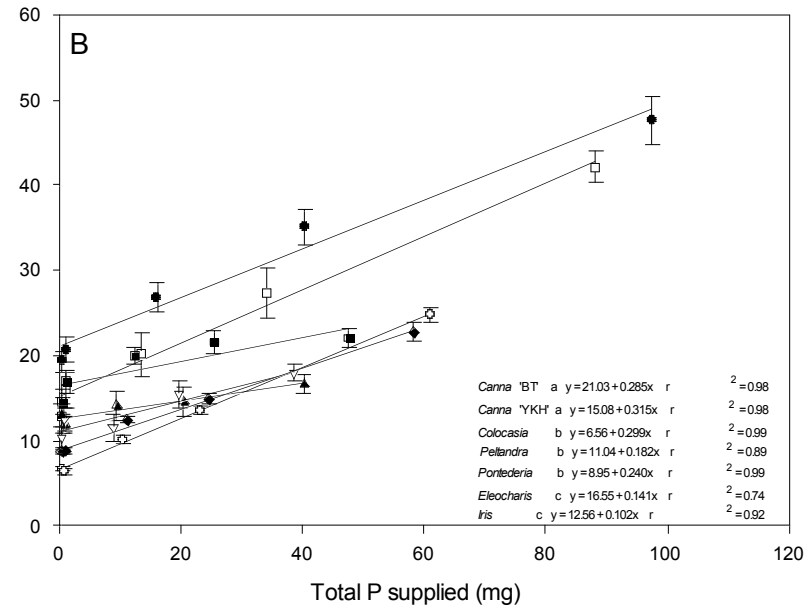
Results and Discussion

Biomass Production

Growth rates for the 7 species increased linearly and were highly correlated with increasing levels of nitrogen and phosphorus over the eight-week period (Figure 2.2A and B), indicating that as plants increased in size and dry weight (DW), they assimilated correspondingly greater amounts of N and P. Due to their higher evapotranspiration rates, *Ca.* 'Bengal Tiger' (*Canna* 'BT') and *C.* '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 $mg \cdot L^{-1}$ 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).

Figure 2.2. The effect of N (A) and P (B) on whole plant dry weight of seven, greenhouse-grown containerized aquatic garden plants over an eight-week period. Five concentrations of modified Hoagland's solution (Table 3, footnote z) were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Vertical bars = ± 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 ($P \leq 0.05$).





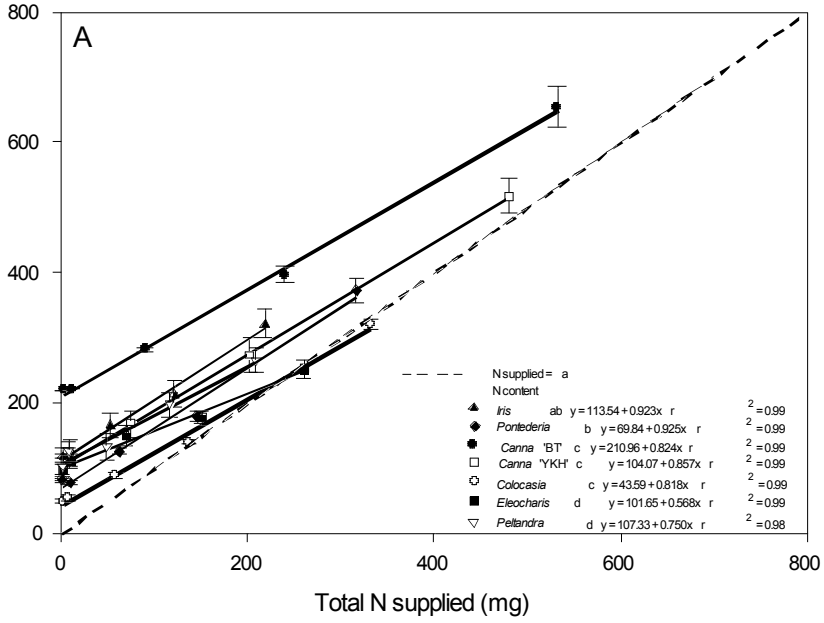
Over the eight-week period the rate of dry weight accumulation in the cannas was greater than the rest of the species (Figure 2.2A and B). *Co. esculenta* 'Illustris' (*Colocasia*), *Pe. virginica* (*Peltandra*) and *Po*. *cordata* 'Singapore Pink' (*Pontederia*) produced biomass more rapidly than *E. dulcis* (*Eleocharis*) and *I.* 'Full Eclipse' (*Iris*). Interestingly, *Peltandra* received the least amount of N and P over the eight-week period but exhibited a higher DW accumulation rate than *Eleocharis* and *Iris* (Figure 2.2A and B). When supplied with the two lowest levels of N and P, the cannas exhibited more severe visual nutrient deficiency symptoms than the other 5 species, which included stunted growth and chlorotic older leaves.

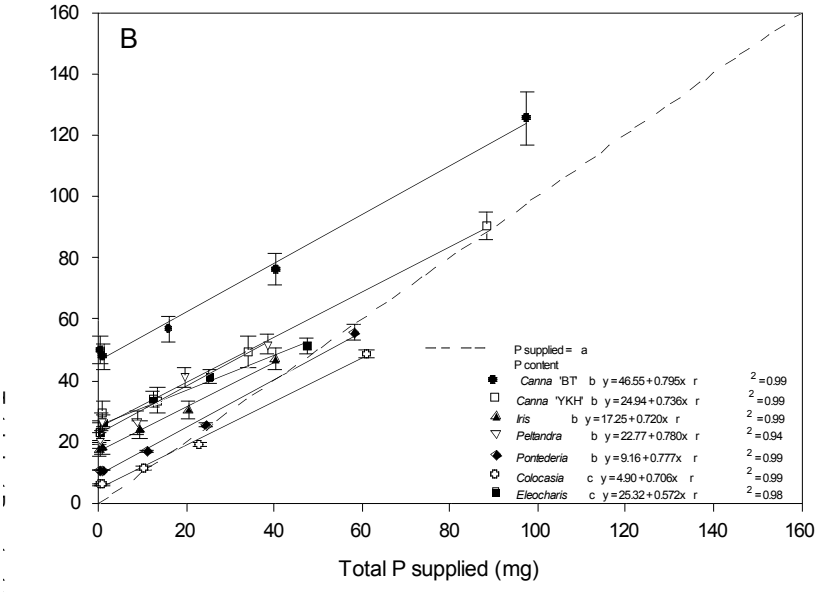
N and P Recovery

Nitrogen and phosphorus 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 phosphorus content of whole plant tissues for all 7 species increased linearly with increasing concentrations of N and P and was highly related to the amount supplied to each species (Figure 2.3A and B). *Canna* 'BT' and 'YKH' received the greatest N amounts; however, their N recovery rates were less than *Iris* and *Pontederia* (Figure 2.3A). 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 (Figure 2.3A).

The cannas also received more P than the other five species over the eight-week period; however, the recovery rate of *Canna* 'BT' and 'YKH' were similar to *Iris*,

Figure 2.3. Nitrogen (A) and phosphorus (B) recovered in whole plant tissues of seven greenhouse-grown aquatic garden species over an eight-week period. Five concentrations of modified Hoagland's solution (Table 1.3, footnote z) were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Vertical bars = ± 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 ($P \leq 0.05$).





Peltandra, and *Pontederia*, which were supplied with less P (Figure 2.3B). *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 eight 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 finding were consistent with other studies that showed an improvement in nutrient removal when plants were present (Tanner et al., 1995; Hunter et al., 2001; Huett et al., 2005). 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), while N depletion may have occurred via 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 confirmed 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 2.3). *Pontederia* and *Iris* shoots had greater N concentrations than roots at treatment levels exceeding 10.4 and 21.6 $mg \cdot L^{-1}$ 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 a N concentration from "five mature leaves from new growth" of a "hybrid canna lily (*Ca.* x*generalis* [*sic*])" 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 four-month microcosm study in Florida,

Table 2.3. Nitrogen (N) and phosphorus (P) concentration and content of shoots and roots of seven aquatic garden plants grown for eight 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. Treatments were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Refer to Appendix A for other mineral concentrations.

Treatment level[z]	Concentration				Content			
	N		P		N		P	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots
	——(mg·g^{-1})——				——(mg)——			
	Canna '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
	Canna '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
	Colocasia esculenta '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

[z]1 = 0.39 N mg·L^{-1}/0.07 P mg·L^{-1}; 2 = 1.75 N mg·L^{-1}/0.18 P mg·L^{-1}; 3 = 10.44 N mg·L^{-1}/1.86 P mg·L^{-1}; 4 = 21.57 N mg·L^{-1}/3.63 P mg·L^{-1}; 5 = 36.81 N mg·L^{-1}/6.77 P mg·L^{-1}; 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 $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2.3. Nitrogen (N) and phosphorus (P) concentration and content of shoots and roots of seven aquatic garden plants grown for eight weeks in a laboratory scale wetland (continued).

Treatment level[z]	Concentration				Content			
	N		P		N		P	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots
	(mg·g^{-1})				(mg)			
Eleocharis dulcis								
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
Louisiana Iris 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
Peltandra virginica								
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**
Pontederia cordata '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

[z]1 = 0.39 N mg·L^{-1}/0.07 P mg·L^{-1}; 2 = 1.75 N mg·L^{-1}/0.18 P mg·L^{-1}; 3 = 10.44 N mg·L^{-1}/1.86 P mg·L^{-1}; 4 = 21.57 N mg·L^{-1}/3.63 P mg·L^{-1}; 5 = 36.81 N mg·L^{-1}/6.77 P mg·L^{-1}; 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 $P \leq 0.05$ and $P \leq 0.01$, respectively.

DeBusk et al. (1995) reported that water canna (*Ca. flaccida* Salisb.) and *Pe. virginica* receiving "enriched" (75.7 $mg \cdot L^{-1}$ N, 29.2 $mg \cdot L^{-1}$ P) and "unenriched" (9.7 $mg \cdot L^{-1}$ N, 1.7 $mg \cdot L^{-1}$ 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 $mg \cdot L^{-1}$ N) was less than our highest N concentration (30.9 $mg \cdot L^{-1}$ 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 $mg \cdot L^{-1}$ P treatments 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 four-fold higher P concentration measured by Nyakang'o and Van Bruggen was likely due 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. quandrangulata* ([Michx.] Roem. & J. Schult.) in South Carolina (Boyd, 1975), but within the range of *E. acuta* R. Br., *E. philippinensis* Svenson, and *E. sphacelata* R. Br. growing in a constructed wetland in Australia (Greenway and Woolley, 1999).

N and P Content

Total nitrogen content/plant [plant dry weight x 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' 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 $mg \cdot L^{-1}$ P. 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% and ≥ 70%, respectively, in shoots. *Pontederia* was the only species that concentration and content followed identical trends. *Peltandra* had ≥ 63% 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 due to 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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CHAPTER III

DIFFERENTIAL NITROGEN AND PHOSPHORUS RECOVERY BY FIVE AQUATIC GARDEN SPECIES IN LABORATORY-SCALE SUBSURFACE CONSTRUCTED WETLANDS

Abstract

Intensive production of container-grown nursery and greenhouse crops in soil-less substrate may result in significant leaching of nutrients and pesticides. The resulting runoff can escape from production areas and negatively impact surface and ground water. Constructed wetlands (CWs) have been shown to be a simple, low-technology method for treating agricultural, industrial, and municipal wastewater. We investigated the nitrogen (N) and phosphorus (P) removal potential by a vegetated, laboratory-scale subsurface flow (SSF) CW system. Over an eight-week period five commercially available aquatic garden plants received a range of N and P (0.39 to 36.81 mg•L^{-1} N and 0.07 to 6.77 mg•L^{-1} P) that spanned the rates detected in nursery runoff. Whole plant dry weight was positively correlated with N and P supplied. Highest N and P recovery rates were exhibited by *Thalia geniculata* f. *rheumoides* Shuey and *Oenenathe javanica* (Blume) DC. 'Flamingo.' *Phyla lanceolata* (Michx.) Greene also had high P recovery rates. The potential exists for using SSF CWs to concomitantly produce aquatic garden plants and attenuate nutrients in a sustainable nursery enterprise.

Introduction

Container production in nursery and greenhouse operations using soilless media involves inputs of fertilizers, growth regulators, insecticides, and fungicides. Repeated

excessive irrigation leads to leaching and loss of nutrients and chemicals in runoff. The presence of nutrients in runoff and concerns of their impact on surface and groundwater quality has undergone increasing interest and scrutiny from the public, environmental groups, governmental agencies, and elected officials. Since its enactment the U. S. Environmental Protection Agency (EPA) has enforced provisions of the Clean Water Act (1972) related to point-source pollution. In 1999, EPA began enforcing the nonpoint source pollution controls specified in section 303(d) of the Clean Water Act, which mandates that all states implement a Total Maximum Daily Load (TMDL) program for all watersheds and bodies of water (U. S. EPA, 2000). A TMDL as defined in Section 303(d)(1)(C) of the Clean Water Act is the maximum amount of pollutant that a waterbody can receive from point and nonpoint sources and still maintain its designated use and value (e.g., drinking water, fish and wildlife habitat, recreation, etc.). The Clean Water Act (U. S. EPA, 1994) lists N and P as potential pollutants of impaired water bodies. Offsite movement of nitrate-nitrogen (NO_3^-) and soluble reactive phosphate ($H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}) from nursery and greenhouse operations may lead to excessive algal and aquatic plant growth in surface waters, resulting in accelerated eutrophication. In general, freshwater systems are P-limited and more prone to P inputs, while N often limits primary production in estuarine and marine environments (Carpenter et al., 1998).

The maximum contaminant level for NO_3^- in drinking water is 10 $mg \cdot L^{-1}$ (National Academy of Sciences, 1977). No federal limits on P contamination in freshwater have been established due to variations in size, hydrology, and depth of rivers and lakes, and regional differences in P impacts. However, U. S. EPA recommends that

total P not exceed 0.05 mg•L^{-1} in any streams discharging into lakes or reservoirs and 0.10 mg•L^{-1} in streams or other flowing waters that do not (U. S. EPA, 1986).

Fertigation runoff in greenhouse crop production can contain 100 mg•L^{-1} NO_3-N (Wood et al., 1999). In nursery crop production, nursery runoff NO_3-N concentrations range from 0.1 to 135 mg•L^{-1} (Alexander, 1993; Taylor et al., 2006; Yeager, et al., 1993) and P levels from 0.01 to 20 mg•L^{-1} P (Alexander, 1993; Headley et al., 2001; James, 1995; Taylor et al., 2006). These cited N and P runoff ranges could be higher or lower in other nursery and greenhouse crop production systems.

Recently TMDLs of nutrients in agricultural runoff were adopted by environmental regulatory agencies in every state (Yeager, 2006). This follows a trend where state governments have been passing more stringent laws and regulations assessing and regulating nonpoint sources of pollutants beyond the scope of the provisions of the Clean Water Act.

CWs have been promoted as an inexpensive, low-technology approach to comply with increasingly stringent environmental regulations regarding the discharge of nonpoint source pollutants in greenhouse and nursery production (Arnold et al., 1999; Berghage et al., 1999). Surface-flow (SF) and SSF CWs are two commonly used wetland designs to treat agricultural wastewater (Berghage et al., 1999; Scholz and Lee, 2005). A SF CW resembles a shallow (0.2-0.8 m) freshwater marsh and generally requires a large land area for wastewater treatment (Kadlec and Knight, 1996). To remediate nursery and greenhouse wastewater, surface area can be reduced with a concomitant increase in depth (~1.25-1.5 m), which promotes anaerobic conditions that facilitate denitrification.

Alternatively, greenhouse and nursery operations constrained by limited production space and expensive land can use a SSF CW, which consists of a lined or impermeable basin filled with a coarse medium, typically gravel, and wetland plants (Hunter et al., 2001; Kadlec and Knight, 1996). Wastewater flows horizontally or vertically below the surface of the media to prevent exposure to humans or wildlife. SSF CWs can be operated in continuous-flow or batch-load treatment modes with varying hydraulic residence times (Burgoon et al., 1995).

Nitrogen removal from SSF CWs is accomplished primarily by denitrification and plant uptake (Vymazal, 2007). Inorganic or organic P, which has no valency changes during its biotic assimilation or microbial decomposition, is mainly removed via microbial and plant uptake (Vymazal, 2007). Roots and rhizomes support rhizospheric microorganisms by providing colonizing sites, exuding carbohydrates, sugars, amino acids, enzymes, and many other compounds (Rovira, 1969), and oxidizing the rhizosphere (Wießner et al., 2002), which fosters microbial activity.

One of the many factors that control the efficiency of nutrient and bacterial removal in wetlands is vegetation type (Hammer, 1989). Wetland plants have species-specific efficiencies regarding their abilities to aerate water, grow within the constraints of the wetland environment, and remove nutrients and heavy metals (Maschinski, et al., 1999). Previously studied aquatic emergent plants for CWs include reed canarygrass (*Phalaris arundinacea* L.), common reed (*Phragmites australis* [Cav.] Trin. Ex Steud.), reed mannagrass (*Glyceria maxima* [Hartman] Holmb.), softstem bulrush (*Schoenoplectus tabernaemontani* [C. C. Gmel.] Palla), yellow flag (*Iris pseudacorus* L.),

and cattail (*Typha* spp. L.) (Ansola et al., 1995; Hunter et al., 2001; Wolverton et al., 1983). They have not been widely used because of their potential invasiveness. Additionally, their high rates of biomass production necessitates periodic harvesting to prevent the seasonal export of nutrients, particularly P via vegetative decomposition (Hunter et al., 2001).

In this study we investigated a cost-effective approach suggested by Adler et al. (2003): "One way to reduce water treatment costs is to produce a product of value concomitant with treatment of the water." Instead of traditional wetland plants, commercially available aquatic garden plants can be used in a production/remediation system that could generate revenue. Few studies have examined the ability of aquatic garden plants to thrive in SSF CWs and recover nursery runoff rates of nitrogen and phosphorus (Arnold et al., 1999; Holt et. al, 1999; Arnold et al., 2003).

In an earlier study, we investigated the potential of 7 aquatic garden plants to assimilate N and P in a laboratory scale, gravel-based SSF CW system (Polomski et al., 2007). Louisiana Iris hybrid 'Full Eclipse' exhibited the highest N recovery rate, while similar P recovery rates were observed in *Canna* 'Bengal Tiger,' *Canna* 'Yellow King Humbert,' Iris 'Full Eclipse,' *Peltandra virginica* (L.) Schott, and *Pontederia cordata* L. 'Singapore Pink' (Polomski et al., 2007). Our objective was to investigate five additional commercially available aquatic herbaceous emergent garden plants—three upright and two creeping—for their ability to thrive and recover N and P in a laboratory scale wetland system that approximated a SSF CW.

Materials and Methods

Experimental procedures were similar to those described by Polomski et al. (2007); however, an abbreviated description follows with an emphasis on the experimental setup and nutrient solution treatments.

Plant Culture

This greenhouse study was conducted from 2003-2004 in Clemson University's Biosystems Research Complex (lat. 34^{o} N, Clemson, SC, US). Five herbaceous emergent aquatic plants were chosen for their aesthetic features and commercial availability (Table 3.1). Divisions of miniature cattail (*Typha minima* Hoppe), *Rhyncospora colorata* (L.) H. Pfeiffer and *Oenanthe javanica* 'Flamingo' were separated from stock plants (Charleston Aquatic Nursery, Johns Island, SC). Micropropagated plantlets of *Thalia geniculata* f. *rheumoides* were purchased from a commercial tissue culture lab (Agri-Starts II, Apopka, FL). *Phyla lanceolata* (Charleston Aquatic Nursery) was rooted from 7.6 to 10.2 cm long stem cuttings and then individual plants were transplanted into 15-cm diameter containers containing a peat/vermiculite growing substrate (Fafard Germination Mix, Fafard Inc., Anderson, SC). Plants were maintained on the greenhouse bench in water-filled plastic-lined trays and watered and fertilized as needed.

The laboratory subsurface treatment wetland was simulated by two polyethylene containers: a 16.5-cm. diameter "azalea" container filled with pea gravel and placed inside a 16.7-cm diameter aquatic container (2.8-L container with no drainage holes) so their rims were even. An equilibrium isotherm experiment indicated no detectable P-adsorption by the pea gravel (Polomski et al., 2007).

Table 3.1. Species, family, cold hardiness, and description of five commercially available aquatic garden plants examined for their ability to recover runoff rates of N and P (Speichert and Speichert, 2004; USDA, NRCS, 2007; eFloras.org, 2007).

Species	Family	USDA Cold hardiness zone	Description
Oenenathe javanica 'Flamingo'	Apiaceae	5-11	Low-growing Korean native, rainbow water parsley has aromatic pink, white, and green leaves with the aroma of parsley, and grows 15 cm high. White umbels emerge in summer through fall.
Phyla lanceolata	Verbenaceae	5-11	Creeping North American native, lanceleaf frogfruit grows 5-10 cm high, tolerates light foot traffic, and produces tiny white flowers that fade to yellow and then pink; foliage turns reddish-pink in autumn.
Rhyncospora colorata	Cyperaceae	8-11	Native to North America, white-top sedge grows 30-61 cm tall and produces white starlike flowers.
Thalia geniculata f. *rheumoides*	Marantaceae	8-11	Widely distributed in parts of the Americas and W. Africa, red-stemmed alligator flag has reddish-purple petiole, sheath, and pulvinus and bears long arching flower spikes of silvery-purple flowers; grows 0.6 to 3 m tall and 0.6-1.8 m wide.
Typha minima	Typhaceae	3-9	Native to parts of the Middle East and central Asia, miniature cattail reaches a garden height of 30-46 cm; brown marble-sized catkins rise above its 3-6 mm wide blue-green leaves.

Two to four weeks prior to the start of an experiment, 40 to 50 plants of each species or cultivar were removed from their containers, their roots washed free of substrate, weighed, and transplanted into gravel-filled azalea containers. Single plantlets

of *Thalia*, *Oenanthe*, and *Phyla* and 3 each of *Rhyncospora* and *Typha* were planted in each container. After placing the azalea inside the aquatic container, ~1.35 L of a 10% modified Hoagland's solution (21.57 mg•L^{-1} N and 3.63 mg•L^{-1} P) (Hoagland and Arnon, 1950) was added until water appeared at the gravel surface. During acclimation plants were watered every two or three days to maintain water levels just below the gravel surface.

Average daily temperatures, relative humidity, and daily light integral are listed in Table 3.2. A 16-h photoperiod was maintained during the winter months with 1000 W metal halide lights.

Treatments

Five treatment levels of a modified Hoagland's solution ("solution 1" using NO_3-N) contained the following mean concentrations of N and P (mg•L^{-1}): (1) 0.39 N; 0.07 P; (2) 1.75 N; 0.18 P; (3) 10.44 ; 1.86 P; (4) 21.57 N; 3.63 P; and (5) 36.81 N; 6.77 P. These concentrations encompassed the typical range of nutrients found in constructed wetland discharge and nursery runoff, and used in nursery irrigation. The initial pH of the nutrient solution was adjusted to 6.2 with 6 N H_2SO_4.

At the start of the experiment, 30 acclimatized plants were removed from their aquatic containers, flushed with deionized water, and then returned to the aquatic containers that had been emptied and rinsed with deionized water. The appropriate treatment solution was batch-loaded into the containers with plants until it was visible at

Table 3.2. Experiment dates and selected environmental variables (mean ± SE) for the two replicates of each species conducted in the Biosystems Research Complex greenhouses, Clemson University, Clemson, SC.

	Experiment 1			Experiment 2		
Species	Temperature (°C)	Relative Humidity (%)	Daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)	Temperature (°C)	Relative Humidity (%)	Daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)
	17-July-03 to 11-Sep-03			24-Oct-03 to 18-Dec-03		
Oenenathe javanica 'Flamingo'	27.4 ± 0.1	72.9 ± 0.5	21.7 ± 0.5	22.9 ± 0.2	58.3 ± 1.1	10.6 ± 0.6
	22-Jan-04 to 17-Mar-04			20-Jan-04 to 16-Mar-04		
Phyla lanceolata	22.3 ± 0.2	48.6 ± 1.4	11.8 ± 0.9	22.2 ± 0.2	48.6 ± 1.3	11.6 ± 0.9
	12-Sep-03 to 6-Nov-03			28-Oct-03 to 23-Dec-03		
Rhyncospora colorata	24.6 ± 0.2	61.7 ± 1.1	15.9 ± 0.8	22.2 ± 0.2	51.7 ± 1.5	12.0 ± 0.7
	17-Sep-03 to 13-Nov-03			18-Sep-03 to 14-Nov-03		
Thalia geniculata f. *rheumoides*	24.3 ± 0.2	60.9 ± 1.2	17.2 ± 0.9	24.3 ± 0.2	61.0 ± 1.2	17.0 ± 0.9
	22-Jul-03 to 15-Sep-03			28-Oct-03 to 23-Dec-03		
Typha minima	27.2 ± 0.1	72.2 ± 0.6	21.3 ± 0.5	22.8 ± 0.3	57.2 ± 1.1	10.8 ± 0.6

the gravel surface. Six containers without plants (gravel only) received 10.44 and 1.86 $mg \cdot L^{-1}$ N and P, respectively. Thereafter, nutrient solution was supplied every two days to maintain the water level at the gravel surface.

Containers were arranged in a randomized complete block design with 6 replicates. Experiments were repeated twice for each species during the time periods listed in Table 3.2.

Data Collection

During the course of each experiment the volume of nutrient solution supplied to each wetland unit was recorded over the eight-week period. When the experiment was terminated, the above- and below-ground portions of each plant were severed at the gravel surface and weighed. The below-ground portions, which included roots that had grown through the drainage holes of the gravel-filled azalea containers were placed over a screen and washed with tapwater, rinsed with distilled water, and then weighed. Dried roots and shoots (80 ºC to constant dry weight) were ground separately in a Wiley® Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 40-mesh (0.425-mm) screen. N and P tissue concentrations were determined as described by Polomski et al. (2007). To normalize differences in nutrient concentrations as a result of growth differences between treatments, N and P plant tissue nutrient content was calculated by multiplying plant part dry weight by nutrient concentration. Whole plant N and P content was derived by adding above- and below-ground mineral content.

The water that remained in the aquatic containers was sampled and stored at 4 ºC until anion analysis with a Dionex AS50 IC with AS50 autosampler (Dionex Corp., Sunnyvale, CA) to determine the percentage of recovered nutrient ([mg N or P supplied – mg nutrient remaining in solution ÷ mg N or P supplied] x 100).

Statistical Analysis

Data from repetitions of the experiments were pooled because analysis of variance (ANOVA) indicated no significant treatment interactions with replication and block. Regression analyses were performed for each species to describe changes in

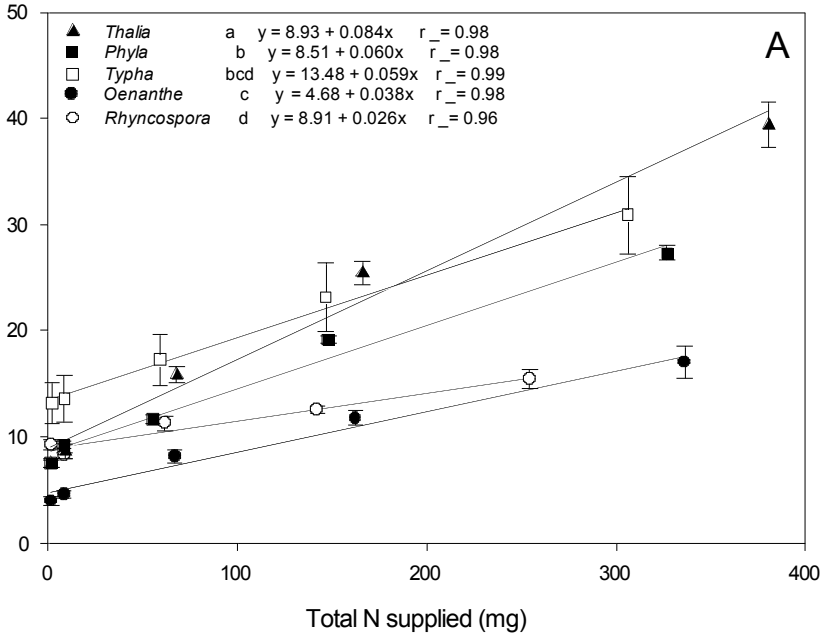
biomass and nutrient recovery relative to N or P supplied. The analysis indicated significant slope for biomass and nutrient uptake efficiency (i.e., the proportion of nutrient applied that is assimilated by the plant) for each species. Comparison of slopes among the species was accomplished using linear contrasts and F tests. Differences between shoot and root concentration means and content means were determined by Student's *t* tests. All analyses were performed with SAS (version 9.1 for Windows; SAS Institute, Cary, NC), and all tests were conducted with $\alpha = 0.05$.

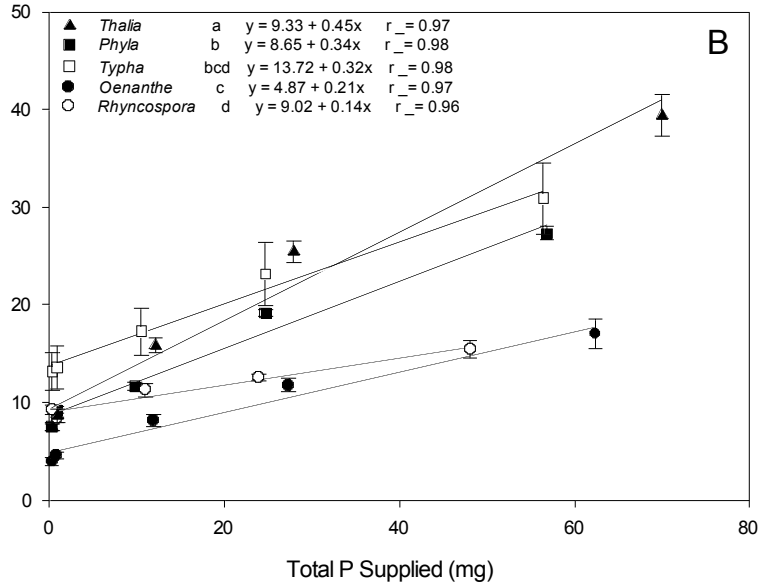
Results and Discussion

Biomass Production

Growth rates increased linearly and were highly correlated with levels of N and P supplied (Figure 3.1A and B). *Thalia* was supplied with greater amounts of N and P than the other species due to its higher evapotranspiration rate. Higher quantities of nutrients resulted in the highest rate of dry weight accumulation. *Rhyncospora* received the least amount of N and P over the eight-week period and had the lowest growth rate compared to *Thalia*, *Phyla*, and *Oenanthe* (Figure 3.1A and B). Gravel-only containers receiving 10.44 and 1.86 $mg \cdot L^{-1}$ N and P, respectively, were supplied with 62 to 86% less N and 52 to 86% less P than planted containers receiving the same level of N and P (data not presented). Although *Oenanthe* and *Phyla* received nearly equal amounts of N and P, *Phyla* exhibited a higher growth rate than *Oenanthe*. When supplied with the two lowest treatment levels of N and P, all species exhibited visual deficiency symptoms that included spindly growth and chlorotic, senescent older leaves. Symptoms were more pronounced in *Thalia* than in the other four species.

Figure 3.1. The effect of N (A) and P (B) on whole plant dry weight of 5 greenhouse-grown containerized aquatic garden plants over an eight-week period. Five concentrations of modified Hoagland's solution (N and P [mg•L^{-1}]: (1) 0.39 N; 0.07 P; (2) 1.75 N; 0.18 P; (3) 10.44 ; 1.86 P; (4) 21.57 N; 3.63 P; and (5) 36.81 N; 6.77 P) were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Vertical bars = ± 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 ($P \leq 0.05$).



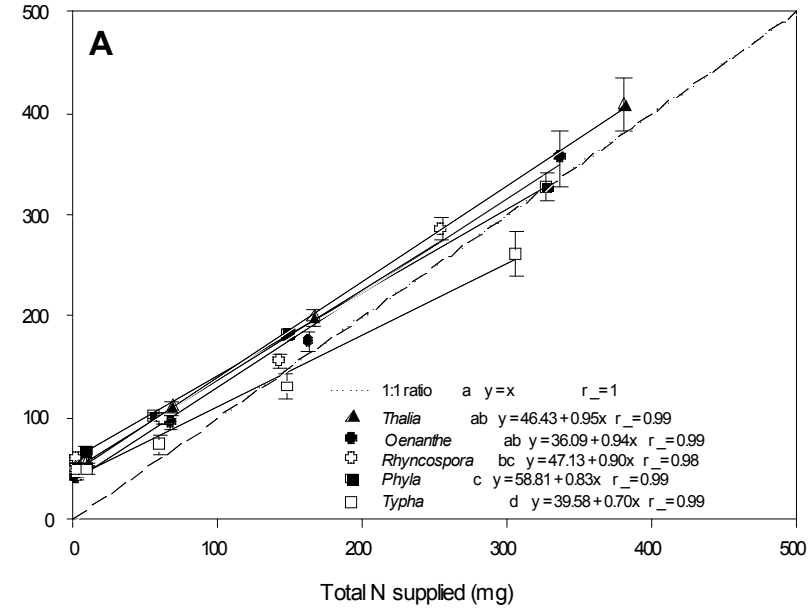


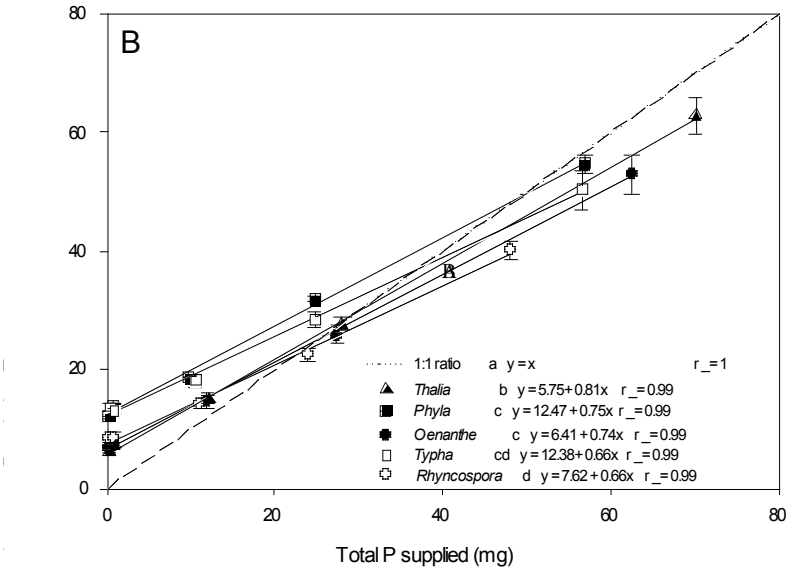
Nitrogen and Phosphorus Recovery

Nitrogen and P recovery rates were determined by comparing the amount of N or P supplied and assimilated in whole plant tissues to an optimal recovery rate where all N or P supplied was recovered in the tissues. Nitrogen and P content of whole plant tissues increased linearly and was highly correlated with the amount supplied to each species (Figure 3.2A and B). Nitrogen recovery rate of *Thalia* and *Oenanthe* was similar to the optimal recovery rate of N. Their N assimilation rates were higher than *Phyla* and *Rhyncospora* (Figure 3.2A). *Typha* had the lowest N recovery rate (Figure 3.2A), contrary to previous research on cattail species (*Typha latifolia* L., *T. angustifolia* L., *T. orientalis* L., and *T. domingensis* Pers.) in CWs (e.g., Scholz and Lee, 2005). Our N source may have affected uptake by *Typha*, since NH_4^+ is the predominant form of inorganic N in acidic, waterlogged, wetland soils (Mitsch and Gosselink, 2007). However, *Typha orientalis* showed no preference for N source in a hydroponics study with four different N sources (2 to 20 mg/L of NO_3^-, NH_4^+, $NH_4NO_3^-$, and urea) (Cary et al., 1984). *T. latifolia* produces optimal growth with either NH_4^+ or NO_3^- at pH 5.0-7.0 (Brix et al., 2002). With NH_4^+ *T. latifolia* has a higher relative growth rate, greater tissue concentration of major nutrients, greater content of adenine nucleotides, and a higher affinity for inorganic N uptake than with NO_3^-. Maximum uptake rate (V_{max}) was highest for NH_4^+ at pH 6.5 and at pH 5.0 for NO_3^- (Brix et al., 2002).

None of the species had P assimilation rates that were similar to the optimal P recovery rate (Figure 3.2B). *Thalia* received more P than the other species and had the highest P recovery rate, followed by *Oenanthe* and *Phyla* (Figure 2.2B).

Figure 3.2. Nitrogen (A) and phosphorus (B) recovered in whole plant tissues of five greenhouse-grown aquatic garden species over an eight-week period. Five concentrations of modified Hoagland's solution (N and P [mg•L^{-1}]: (1) 0.39 N; 0.07 P; (2) 1.75 N; 0.18 P; (3) 10.44 ; 1.86 P; (4) 21.57 N; 3.63 P; and (5) 36.81 N; 6.77 P) were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Vertical bars = ± 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 ($P \leq 0.05$).





Rhyncospora had the lowest P recovery/assimilation rate compared to *Thalia, Oenanthe,* and *Phyla* (Figure 3.2B).

Compared to a similar study with seven other aquatic garden species (Polomski et al, 2007), *Thalia*, *Rhyncospora*, and *Oenanthe* had N recovery rates similar to Louisiana iris hybrid 'Full Eclipse' and *Pontederia cordata* L. 'Singapore Pink.' P recovery rates of *Thalia* were similar to *Canna* 'Bengal Tiger,' *Peltandra virginica* (L.) Schott, and *Pontederia cordata* 'Singapore Pink.'

There were no differences between species or treatment levels in the concentration of N and P remaining in the containers at harvest. Less than 4 and 7% of the original amount of N and P supplied to the plants, respectively, was detected in the remaining solution (data not shown). Of the original amount of N and P supplied to gravel-only containers, 37 to 53% N and 27 to 54% P remained (data not shown). These findings were consistent with other studies that showed an improvement in nutrient removal when plants were present in SSF wetlands (Jing et al., 2002; Huett et al., 2005).

Depletion of P in the gravel-only containers could have resulted from assimilation by the thin film of algae present near the gravel surface and from biofilm--single cells or pools of microorganisms embedded in a matrix of microbial-derived polymers attached to the gravel substrate (Zhang and Bishop, 1994). Phosphorus precipitation was highly unlikely because the pH was not alkaline enough (mean pH of 7.1). Nitrogen depletion may have occurred via denitrification processes.

Nitrogen and Phosphorus Concentration

To characterize differences in N and P tissue accumulation among species, we reported concentration of tissue nutrients in accordance with typical wetland plant nutrient uptake and mass balance studies. Nitrogen concentration in roots exceeded the amount in shoots at every level of N supplied for *Phyla* (Table 3.3). A similar trend was observed with *Oenanthe* at concentrations $\leq$ 21.57 mg•L^{-1} N. However, at the highest treatment level, N concentration was comparable between roots and shoots (Table 3.3). Similar results were reported for *Oenanthe javanica* receiving 16.8 mg•L^{-1} and 33.6 mg•L^{-1} N in sand culture (Wang et al., 2002) and *Oenanthe sarmentosa* sampled from agricultural drainage waterways in central California (Rejmankova, 1992). Similar to *Oenanthe sarmentosa*, more biomass was allocated in *O. javanica* to the aboveground than belowground plant parts with increasing levels of nutrients (data not presented). This preferential allocation of nutrients to belowground parts rather than aboveground parts in response to reduced nutrient status commonly occurs in plants growing in infertile habitats (Chapin III, 1980).

Typha and *Thalia* had higher N concentration in the shoots than the roots at levels $\geq$ 0.39 and $\geq$ 1.75 mg•L^{-1} N, respectively, similar to the trend that *Canna* 'Yellow King Humbert,' *Colocasia esculenta* (L.) Schott var. *antiquorum* (Schott) Hubbard & Rehd. 'Illustris,' and *Peltandra virginica* exhibit (Polomski et al., 2007). Phosphorus concentration in *Thalia* and *Phyla* was highest in shoots at every treatment level, whereas the highest P concentration in *Typha* was in roots at every treatment level.

Contrary to *Typha minima*, N concentration of *T. angustifolia* roots and rhizomes

Table 3.3. Nitrogen (N) and phosphorus (P) concentration and content of shoots and roots of five aquatic garden plants grown for eight weeks in a laboratory scale wetland and receiving 5 treatment levels of N or P from a modified Hoagland's nutrient solution. Values are means of 12 plants. Treatments were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Refer to Appendix B for other mineral concentrations.

Treatment level[z]		Concentration				Content			
		N		P		N		P	
N	P	shoots	roots	shoots	roots	shoots	roots	shoots	roots
($mg \cdot L^{-1}$)		($mg \cdot g^{-1}$)				(mg)			
					Oenanthe				
0.39	0.07	10.30	11.88**	1.63	1.48	29.826**	11.667	5.100**	1.552
1.75	0.18	10.22	11.91**	1.69*	1.42	35.267**	12.885	6.136**	1.596
10.44	1.86	11.43	14.54**	1.80	2.04	79.857**	16.443	12.666**	2.133
21.57	3.63	14.82	16.13*	2.23	2.01	149.792**	26.137	22.609**	3.222
36.81	6.77	21.12	21.83	3.28**	2.40	320.046**	36.374	48.832**	3.938
					Phyla				
0.39	0.07	7.30	9.49**	1.69*	1.46	41.507**	16.969	9.679**	2.576
1.75	0.18	6.60	10.30**	1.67**	1.30	47.576**	20.341	11.313**	2.596
10.44	1.86	8.32	11.65**	1.70**	1.30	80.199**	22.128	16.059**	2.498
21.57	3.63	9.13	12.89*	1.70**	1.42	153.663**	28.997	28.624**	3.234
36.81	6.77	11.58	15.55**	2.03**	1.82	283.853**	43.879	49.542**	5.089
					Rhyncospora				
0.39	0.07	7.10*	5.63	0.85	1.05	37.190**	21.826	4.486	4.045
1.75	0.18	7.76**	5.58	0.98	1.12	36.185**	19.810	4.570	3.999
10.44	1.86	10.02**	7.44	1.29	1.32	68.229**	30.173	8.820**	5.294
21.57	3.63	13.00	11.43	1.81	1.81	111.072**	44.491	15.514**	6.992
36.81	6.77	18.03	21.46**	2.58	2.83	206.837**	79.698	29.594**	10.362
					Thalia				
0.39	0.07	6.83	6.48	0.89**	0.73	30.732**	19.312	3.986**	2.180
1.75	0.18	6.77*	6.19	0.95**	0.71	35.124**	20.851	4.825**	2.387
10.44	1.86	7.48**	6.10	1.06**	0.78	73.666**	36.541	10.407**	4.652
21.57	3.63	8.58**	6.63	1.21**	0.89	133.335**	65.418	18.770**	8.793
36.81	6.77	11.39**	8.45	1.80**	1.24	288.997**	119.308	45.192**	17.631

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

Table 3.3. Nitrogen (N) and phosphorus (P) concentration and content of shoots and roots of five aquatic garden plants grown for eight weeks in a laboratory scale wetland and receiving 5 treatment levels of N or P from a modified Hoagland's nutrient solution. Values are means of 12 plants (continued).

Treatment level		Concentration				Content			
		N		P		N		P	
N	P	shoots	roots	shoots	roots	shoots	roots	shoots	roots
($mg \cdot L^{-1}$)		($mg \cdot g^{-1}$)				(mg)			
					Typha				
0.39	0.07	9.30**	6.60	1.19	2.00**	25.741	33.821*	3.255	10.397**
1.75	0.18	9.87**	6.91	1.33	2.02**	27.903	31.925	3.707	9.454**
10.44	1.86	11.85**	7.72	1.27	2.40**	45.246	41.752	4.888	13.213**
21.57	3.63	14.80**	9.28	1.58	2.72**	89.575**	61.797	9.749	18.672**
36.81	6.77	20.93**	13.04	2.23	4.00**	204.108**	91.845	21.514	28.708*

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

(Steinbachova-Vojtiskova et al., 2006) and *T. latifolia* rhizomes (Cizkova-Koncalova et al., 1996) increases with increasing nutrient availability in contrast to shoots. *T. minima* shoot N concentration was similar to *T. angustifolia* shoot N at comparable N treatment levels (Steinbachova-Vojtiskova et al., 2006); however, root and rhizome N concentration of *T. angustifolia* exceeded the concentration of *Typha minima*. This discrepancy could be explained by the diminutive size of *T minima* and the propensity of *T. angustifolia* to allocate resources to belowground structures, which contributes to its ability to thrive and compete in eutrophic habitats (Steinbachova-Vojtiskova et al., 2006). *T. angustifolia* shoot dry weight increases and root dry weight decreases with increasing nutrient availability (Steinbachova-Vojtiskova et al. (2006), similar to *T. minima* (data not presented).

In natural stands of *Typha latifolia* from Aiken, SC (Boyd, 1978), whole plant N and P concentrations were 1.7- and 2.3-fold less, respectively, than those of *Typha minima* receiving the lowest treatment level in our study. Boyd (1978) expected these concentrations to be 1.5 to 2 times higher if *T. latifolia* received nutrient-rich effluent.

Breen (1990) evaluated *Typha orientalis* in an experimental wetland system in Australia comprised of 10 L polytethylene buckets with gravel (3-7 mm diameter). Mean influent nutrient concentration was 31.83 mg•L^{-1} total N and 11.47 mg•L^{-1} P during the 50-day experiment. Above- and belowground tissue N values of *Typha orientalis* was comparable to *Typha minima* at the 10.44 mg•L-1 N treatment level. Phosphorus rhizome and root concentrations of *Typha orientalis* were similar to *Typha minima* at our highest P treatment level, but above-ground growth of *Typha orientalis* contained twice as much P as *T. minima* at our highest treatment level. Cary and Weerts (1984) grew *Typha orientalis* for 7 weeks hydroponically and the nutrient solution was replaced every 3.5 days. Top-growth N and P concentrations of *T. orientalis* receiving 40 mg•L-1 N and 10 mg•L-1 P were similar to *T. minima* at our highest treatment level.

Rhyncospora shoots had a higher N concentration than roots at nutrient levels $\leq$ 10.4 mg•L^{-1} N, but N root concentration exceeded N shoot concentration at 36.8 mg•L^{-1} N. There were no differences in P between the shoots and roots of *Rhyncospora* at any treatment level. No trend was observed with *Oenanthe*. However, our P concentrations in *Oenanthe* shoots were within the range reported by Wang et al. (2002).

N and P Content

Nitrogen content of *Oenanthe*, *Phyla*, *Rhyncospora* and *Thalia* shoots was > 61% higher than roots at every N treatment level (Table 3.3). Similar sink strength of shoots was reported for Louisiana iris 'Full Eclipse' and *Pontederia cordata* 'Singapore Pink' (Polomski et al., 2007).

Typha roots were a dominant N sink at 0.39 mg•L^{-1} N treatment level, containing 57% more N in roots than shoots; however, at the two highest treatment levels, shoots stored 59% and 69% more N, respectively, than roots. A similar change in sink strength with increasing levels of N was observed with two canna cultivars (*Canna* 'Bengal Tiger' and 'Yellow King Humbert') and *Colocasia esculenta* var. *antiquorum* 'Illustris,' (Polomski et al., 2007).

Phosphorus content of *Oenanthe, Phyla,* and *Thalia* was greater in shoots than roots at every treatment level. *Oenanthe* and *Phyla* shoot P exceeded 86% in shoots at treatment levels ≥ 1.86 mg•L^{-1} P, similar to Louisiana iris 'Full Eclipse,' (Polomski et al., 2007). *Thalia* shoots contained between 65 and 69% more P compared to roots at every treatment level, similar to *Pontederia cordata* 'Singapore Pink.' P concentration and content followed identical trends in *Thalia* and *Phyla* at each treatment level, similar to *Pontederia* 'Singapore Pink' (Polomski et al., 2007). In contrast, *Typha* P root content followed a similar trend to P root concentration: P root content was 57 to 61% greater than shoot P at every treatment level.

There were no statistical differences between *Rhyncospora* shoot and root P content at the two lowest treatment levels, but shoot P exceeded root P at treatment levels

$\geq$ 1.86 mg•L^{-1} P. This partitioning of P to shoots instead of roots with increasing levels of P was also observed in *Canna* 'Bengal Tiger' and *Colocasia esculenta* var. *antiquorum* 'Illustris' (Polomski et al., 2007).

Taxa that preferentially allocate nutrients to aboveground biomass allow for the harvesting and removal of topgrowth. Continuous and longterm removal of excess P from CWs can be ensured by regularly harvesting pollution-tolerant species (Jing et al., 2001). In nursery/greenhouse production systems, container-grown aquatic garden plants receiving runoff channeled into nutrient attenuation/production CW beds can also be "harvested" to remove nutrients from the system. Removal of entire plants avoids P export to outflow and downstream environments from senescent, decomposing tissues (Hunter et al., 2001). Plants with highly efficient N and P recovery rates such as *Thalia* and *Oenanthe* can be placed at the discharge end of a CW to "polish" the effluent. Also, they can be located at the inflow end of CWs because of their ability to assimilate high N and P concentrations. *Thalia*, *Oenanthe*, and *Phyla* may also be suited for SSF CWs in greenhouse production systems because of their ability to assimilate high volumes of nutrient-rich water, which reduces the amount of effluent that must be discarded.

The commercial value of aquatic garden plants offsets their production costs, which offers producers a sustainable, cost-effective and low maintenance remediation solution compared to conventional wastewater treatment technologies. Their usefulness could be expanded to other phytoremediation applications depending on the outcome of additional research assessing their ability to assimilate pesticides (e.g., Fernandez et al., 1999) and other anthropogenic pollutants (i.e., hydrocarbons, and metals) (e.g., Fritioff

and Greger, 2003). The aesthetic features of aquatic garden plants create markets and opportunities in commercial and residential landscape applications, such as infiltration trenches (i.e., basins and rain gardens), retention ponds, and wet or dry detention basins.

Direct comparison of N and P recovery by the aquatic garden plants in this study with other investigations is precluded by differing hydraulic characteristics, such as retention time, water level depth, and wastewater loading, along with differences in species compositions and densities, media, and design and size of the systems. Nevertheless, the results support the use of aquatic garden plants as aesthetic and economically viable alternatives to traditional, obligate wetland plants in CWs and the need for further investigation to optimize species selection, cycling time, and production system design.

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CHAPTER IV

NITROGEN AND PHOSPHORUS REMEDIATION BY THREE FLOATING AQUATIC MACROPHYTES IN GREENHOUSE-BASED LABORATORY-SCALE SUBSURFACE CONSTRUCTED WETLANDS

Abstract

In the greenhouse and container nursery production industry there is potential for runoff of nitrogen (N) and phosphorus (P), which may contaminate surface and groundwater. Since the 1950s constructed wetlands (CWs), as a simple, low-technology method, have been shown to effectively treat agricultural, industrial, and municipal wastewater. We investigated the N and P attenuating potential of three floating hydrophytes planted in a laboratory-scale subsurface flow (SSF) CW system. Over an eight-week period plants were supplied with N and P (0.39 to 36.81 mg•L^{-1} N and 0.07 to 6.77 mg•L^{-1} P) that spanned the rates detected in nursery runoff between the discharge and inflow locations of a commercial nursery currently employing CWs. Whole plant dry weight was positively correlated with N and P supplied. Highest N recovery rates were exhibited by water hyacinth (*Eichhornia crassipes* [Mart.] Solms.) and water lettuce (*Pistia stratiotes* L.) P recovery rates were similar for water hyacinth, water lettuce, and dwarf redstemmed parrotfeather (*Myriophyllum aquaticum* [Vell.] Verdc.). These floating hydrophytes can be cultivated in a SSF CW to remediate runoff losses of N and P. The possibility exists for integrating them into a polycultural remediation system that includes emergent aquatic macrophytes for processing and polishing nursery/greenhouse wastewater.

Introduction

Irrigation of nursery and greenhouse container crops may lead to leaching and loss of fertilizers and other agricultural chemicals. This can pose a threat to groundwater and result in surface water contamination. Runoff containing nitrate-nitrogen (NO_3^--N) and soluble reactive phosphate ($H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}) may lead to excessive algal and aquatic plant growth in surface waters, resulting in accelerated eutrophication, primarily in freshwater streams, rivers, lakes, and reservoirs (Carpenter et al., 1998).

The U. S. Environmental Protection Agency (U. S. EPA) has established the maximum contaminant level for NO_3^--N in drinking water to be 10 mg•L^{-1} (U. S. EPA 1986). No federal limits on P contaminant levels in freshwater exist; however, the U. S. EPA recommends that total P not exceed 0.10 mg•L^{-1} in streams or other flowing waters and 0.05 mg•L^{-1} in any streams that enter lakes or reservoirs (U. S. EPA 1986).

Greenhouse crop production may result in NO_3-N runoff levels of 100 mg•L^{-1} NO_3-N (Wood et al. 1999). Nitrate-nitrogen concentrations in nursery crop runoff can range from 0.1 to 135 mg•L^{-1} (Alexander 1993; Yeager et al., 1993; Taylor et al., 2006) and P concentrations from 0.01 to 20 mg•L^{-1} P (Alexander, 1993; James, 1995; Taylor et al., 2006). To reduce the discharge levels of these nonpoint source pollutants and to comply with increasingly stringent environmental regulations at state and federal levels, CWs have been promoted as inexpensive, low-technology alternatives to conventional water treatment systems. Similar to natural wetlands, CWs treat wastewater with physicochemical and biological processes that involve vegetation, soils, and associated microbial populations in a controlled environment. These engineered wetlands are

defined by their vegetation: free-floating, floating-leaved, emergent, and submerged plants (Vymazal, 2007). In temperate regions emergent macrophytes are commonly used in surface-flow (SF) and SSF CWs to treat agricultural wastewater (Arnold et al., 1999; Berghage et al., 1999; Taylor et al., 2006). Due to the large land area required by typical SF CWs and the concomitant loss of production area, SSF CWs have been recommended as a viable alternative for greenhouse and nursery water treatment (Arnold et al., 1999; Berghage et al., 1999).

In tropical and subtropical regions free-floating hydrophytes are the dominant vegetation in CWs because of their ability to overwinter (Nahlik and Mitsch, 2006). Details of floating aquatic plant CWs are described by DeBusk and Reddy (1987) and Vymazal et al. (1998). Many studies have documented their ability to remediate various anthropogenic pollutants that include nutrients (Gopal, 1987; Vymazal, 2007), herbicides (Wilson et al., 2001; Knuteson et al., 2002), heavy metals (Odjegba and Fasidi, 2004; Liao and Chang, 2004; Padmavathiamma and Li, 2007) and antibiotics (Gujarathi et al., 2005). The high rates of biomass production by floating hydrophytes necessitates periodic harvesting to prevent the export of nutrients, particularly P, via vegetative decomposition and to maintain open water areas to permit increased oxygen exchange (Masifwa et al., 2004; Kadlec, 2005).

Recently, Polomski et al. (2007) proposed a sustainable nutrient remediation strategy that involves the production of economically important emergent macrophytes in a SSF CW that remediates wastewater runoff. The objective of this study was to take an unconventional approach and determine the ability of water hyacinth, water lettuce, and

parrotfeather, to thrive and recover nursery runoff levels of N and P in a similarly constructed laboratory-scale subsurface CW system.

Materials and Methods

Experimental procedures were similar to those described by Polomski et al. (2007). However, a brief description follows with an emphasis on the experimental setup and nutrient solution treatments.

Plant Characterization and Culture

This greenhouse study was conducted from 2003-2004 at Clemson University's Biosystems Research Complex (Clemson, SC, USA; latitude 34°40'8"; longitude 82°50'40"). Water hyacinth, water lettuce, and parrotfeather were selected for their remediating ability and their commercial importance as biological filters in water gardens (Speichert and Speichert, 2004). Water hyacinth is a free-floating plant comprised of a rosette of petiolate leaves, an attractive purple inflorescence, and extensive submerged roots (Gopal, 1987). Despite its free-floating habit, water hyacinth can also root in substrate, which has been postulated as an ancestral trait (Gopal, 1987). Water hyacinth rapidly propagates vegetatively and sexually, although vegetative propagation via fragmentation is the primary form of reproduction. The free-floating, stoloniferous water lettuce produces a rosette of light to lime-green velvety leaves; it can reach a mature height of 30.5 cm (Speichert and Speichert, 2004). It reproduces by offsets that grow from the base of the mature plant. Dwarf redstemmed parrotfeather is a compact selection with bright red prostrate or ascending stems bearing whorls of gray-green

feathery leaves (Speichert and Speichert, 2004). This creeping emergent roots freely in floating mats or anchored in substrate where it reproduces primarily by stem fragmentation (Sytsma, 1989).

Water lettuce and dwarf red-stemmed parrotfeather (Charleston Aquatic Nursery, Johns Island, SC) were floated in tapwater-filled 3.8 L aquatic pots, fertigated with 20-20-20 (Peter's Professional®) water-soluble fertilizer as needed, and maintained in the greenhouse. Water hyacinth stock plants were collected from drainage canals near Cape Coral, Florida, U.S., transferred to 60 L containers and fertigated as needed with 20-20-20 water soluble fertilizer.

Two to four weeks prior to the start of an experiment, 40 to 50 plants were removed from their containers, their roots washed in running tapwater to remove microalgae, and weighed. They were transplanted into the simulated laboratory subsurface CW comprised of two polyethylene pots: a 16.5-cm. diameter "azalea" pot filled with pea gravel and placed inside a 16.7-cm diameter aquatic pot (3.8 L pot with no drainage holes) so their rims were even. Single ramets (vegetatively produced plants) of water hyacinth, individual plantlets of water lettuce, and five 14 cm long rooted stem fragments of parrotfeather were planted in each pot. After fitting the azalea pot into the aquatic pot, ~ 1.350 L of a 10% modified Hoagland's solution (21.57 $mg \bullet L^{-1}$ N and 3.63 $mg \bullet L^{-1}$ P) (Hoagland and Arnon, 1950) was added to each pot until water appeared at the gravel surface. During the acclimation period, plants were watered every two or three days to maintain the water level just below the gravel surface. The average daily temperatures, relative humidity, and daily light integral are listed in Table 4.1. A 16:8 h

light:dark photoperiod was maintained during the winter months with 1000 W metal halide lights.

Nitrogen and Phosphorus Treatment Solutions

Five treatment levels of 0.1, 1, 5, 10 and 20% modified Hoagland's solution ("Solution 1" using NO_3-N) were prepared and contained the following mean concentrations of N and P ($mg \bullet L^{-1}$): (1) 0.39 N; 0.07 P; (2) 1.75 N; 0.18 P; (3) 10.44 ; 1.86 P; (4) 21.57 N; 3.63 P; and (5) 36.81 N; 6.77 P. These concentrations encompassed the typical range of nutrients found in nursery CW discharge and nursery runoff, and used in nursery irrigation. The initial pH of the nutrient solution was adjusted to 6.2 with 6 N H_2SO_4.

At the start of the experiment, 30 acclimatized plants and 6 gravel-only pots were removed from their aquatic containers, flushed with deionized water, and then returned to the aquatic pots that had been emptied and rinsed with deionized water. 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 $mg \cdot L^{-1}$ N and P, respectively. Thereafter, nutrient solution was supplied every two days to maintain the water level at the gravel surface. Containers were arranged in a randomized complete block design with 6 replicates. Experiments were replicated twice for each species during the time periods listed in Table 4.1.

Table 4.1. Experiment dates and selected environmental variables (mean ± SE) for the two replicates of each species conducted in the Biosystems Research Complex greenhouses, Clemson University, Clemson, SC.

	Experiment 1			Experiment 2		
Species	Temperature (ºC)	Relative humidity (%)	Daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)	Temperature (ºC)	Relative humidity (%)	Daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)
	25-Jun-03 to 20-Aug-03			9-Sept-03 to 3-Nov-03		
Eichhornia crassipes	27.3 ± 0.07	72.3 ± 0.3	25 ± 1	24.5 ± 0.2	61.5 ± 1.1	19 ± 1
	26-Jun-03 to 21-Aug-03			20-Jan-04 to 15-Mar-04		
Myriophyllum aquaticum	27.3 ± 0.07	74.5 ± 0.3	22 ± 1	22.2 ± 0.2	48.0 ± 1.3	12 ± 1
	17-July-03 to 11-Sep-03			24-Oct-03 to 18-Dec-03		
Pistia stratiotes	24.6 ± 0.2	61.0 ± 1.1	15 ± 1	24.3 ± 0.2	61.0 ± 1.2	16 ± 1

Plant and Water Analysis

Over the course of each experiment the volume of nutrient solution supplied to each wetland unit was recorded over the eight-week period. When the experiment was terminated, each plant was severed at the gravel surface and the above- and below-ground portions were weighed. The below-ground 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 tapwater, rinsed with distilled water, and then weighed. Dried roots and shoots (80 ºC to constant dry weight) were ground separately in a Wiley Mill® (Thomas Scientific, Swedesboro, NJ) to pass through a 40-mesh (0.425-mm screen). N and P tissue concentrations were determined as described by Polomski et al. (2007). N and P content was calculated by multiplying plant part dry weight by nutrient concentration. Whole plant N and P content was derived from combining above- and below-ground mineral content.

The water that remained in the aquatic pots was sampled and stored at 4 ºC prior to anion analysis with a Dionex AS50 IC with AS50 autosampler (Dionex Corp., Sunnyvale, CA). Percentage of recovered nutrient was determined with the following equation: (mg N or P supplied – mg nutrient remaining in solution ÷ mg N or P supplied) x 100.

Statistical Analysis

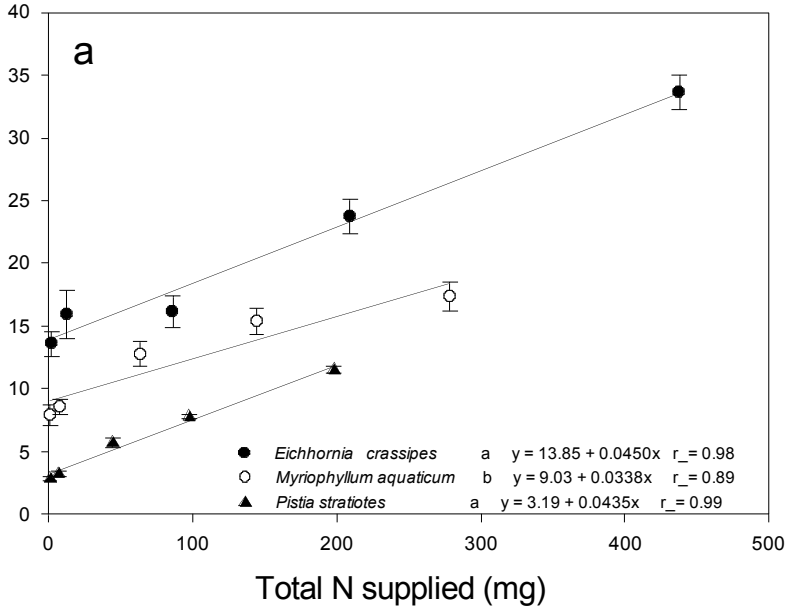
Data from both replicated experiments were pooled because analysis of variance indicated no significant treatment interactions with rep and block. Changes in biomass and nutrient recovery relative to N or P supplied for each species was determined by regression analyses. For each species the analyses indicated significant slope for biomass and nutrient uptake efficiency (i.e., amount of nutrient supplied that is assimilated by the plant). Linear contrasts and F tests compared slopes among the species. Differences between shoot and root concentration means and content means of each species were determined by Student's *t* tests. All analyses were performed with SAS (version 9.1 for Windows; SAS Institute, Cary, NC), and all tests were conducted with $\alpha = 0.05$.

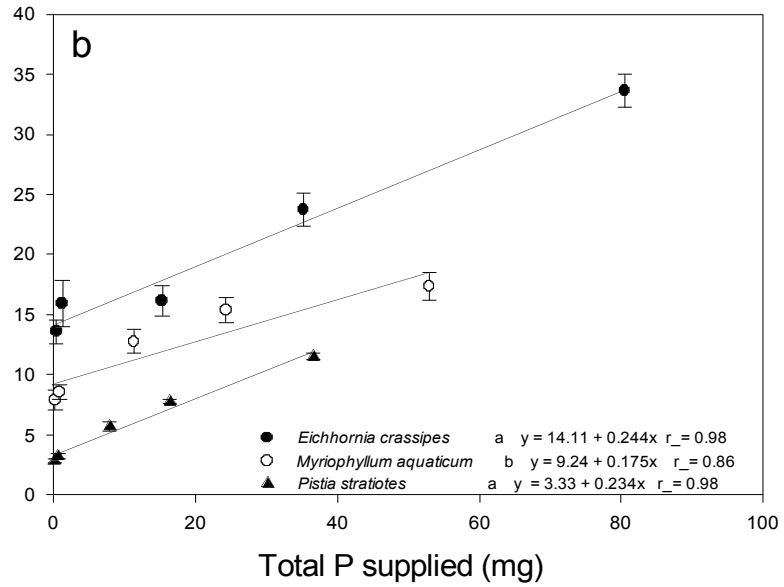
Results and Discussion

Biomass Accumulation

Over the eight-week period the growth rates of the 3 species increased linearly and were highly correlated with increasing levels of nitrogen and phosphorus (Figure 4.1A and B). Due to its higher evapotranspiration rate (Gopal, 1987), water hyacinth was supplied with greater amounts of N and P than the other two species, which yielded the

Figure 4.1. The effect of N (a) and P (b) on whole plant dry weight of three, greenhouse-grown floating hydrophytes growing in a laboratory scale subsurface flow constructed wetland over an eight-week period. Five concentrations of modified Hoagland's solution (N and P [mg•L^{-1}]: (1) 0.39 N; 0.07 P; (2) 1.75 N; 0.18 P; (3) 10.44 ; 1.86 P; (4) 21.57 N; 3.63 P; and (5) 36.81 N; 6.77 P) were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Vertical bars represent standard error of N or P content. Data points are means of 12 plants. Slopes of regression lines were compared using linear contrasts and F tests; species with different letters have significantly different slopes ($P \leq 0.05$).





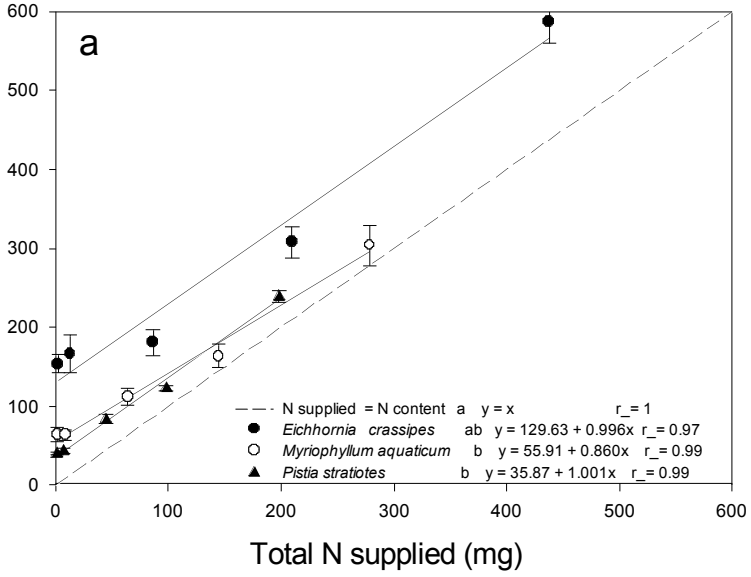
highest rate of dry weight accumulation. Water lettuce received the least amount of N and P over the eight-week period but had a similar growth rate to water hyacinth (Figure 4.1A and B). Parrotfeather had the lowest growth rate compared to water hyacinth and water lettuce. Gravel-only pots receiving 10.44 and 1.86 $mg \cdot L^{-1}$ N and P, respectively, were supplied with 62 to 86% of N and 52 to 86% of P than planted pots receiving the same level of N and P (data not presented).

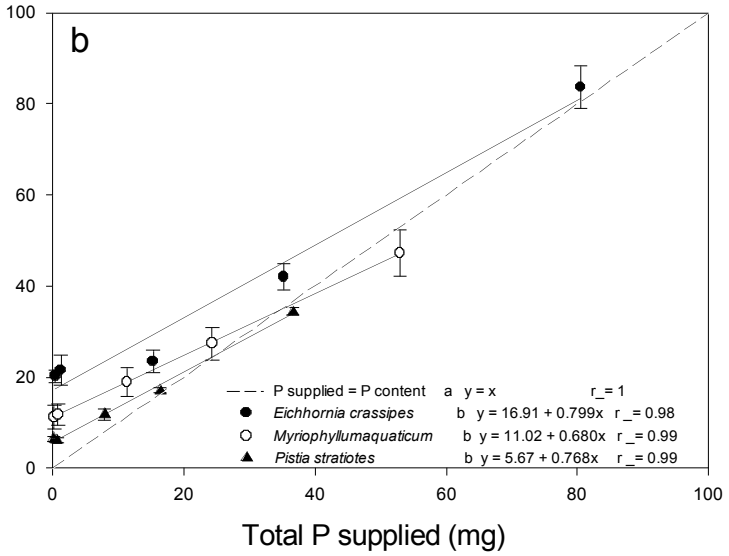
At the lowest treatment level, all species exhibited visual nutrient deficiency symptoms that included marginal to complete foliar necrosis, chlorotic, senescent leaves, and spindly growth. Some water hyacinths produced inflorescences, which was not unexpected since water hyacinth has been reported to survive and grow under a wide range of water nutrient concentrations as low as 0.05 $mg \cdot L^{-1}$ nitrogen supplied either as nitrate (Shiralipour et al. 1981) or ammonia (Tucker 1981) and 0.1 $mg \cdot L^{-1}$ P, which Haller et al. (1970) determined as the lower critical level for growth of water hyacinth in a hydroponic environment.

N and P Recovery

Nitrogen and phosphorus recovery rates of the three species were evaluated by comparing the amount of N or P supplied and assimilated in whole plant tissues to an optimal recovery rate where 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 three species increased linearly with increasing concentrations of N and P and was highly correlated with the amount supplied to each species (Figure 4.2A and B). The N recovery rate of water hyacinth and water lettuce was similar to the optimal recovery rate

Figure 4.2. Nitrogen (a) and phosphorus (b) recovered in whole plant tissues of three greenhouse-grown floating hydrophytes growing in a laboratory scale subsurface flow constructed wetland over an eight-week period. Five concentrations of modified Hoagland's solution (N and P [mg•L^{-1}]: (1) 0.39 N; 0.07 P; (2) 1.75 N; 0.18 P; (3) 10.44; 1.86 P; (4) 21.57 N; 3.63 P; and (5) 36.81 N; 6.77 P) were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Vertical bars represent standard error of N or P content. Data points are means of 12 plants. Dashed line represents hypothetical 100% recovery rate. Slopes of regression lines were compared using linear contrasts and F tests; species with different letters have significantly different slopes ($P \leq 0.05$).





of N (mg N supplied = mg N in tissues) and higher than the N assimilation rate of dwarf red-stemmed parrotfeather (Figure 4.2A). Compared to similar studies with herbaceous emergent aquatic plants, water hyacinth and water lettuce had N uptake efficiencies similar to Louisiana iris hybrid 'Full Eclipse,' *Pontederia cordata* 'Singapore Pink,' *Thalia geniculata* f. *rheumoides* Shuey, *Rhyncospora colorata* (L.) H. Pfeiffer, and *Oenenathe javanica* (Blume) DC. 'Flamingo' (Polomski et al., 2007; 2008).

None of the three species had P assimilation rates that were similar to the optimal P recovery rate (Figure 4.2 B). The P recovery rates were similar for water hyacinth, parrotfeather, and water lettuce. Their P recovery rates were similar to *Canna* x *generalis* Bailey (pro sp.) 'Bengal Tiger,' *Peltandra virginica* (L.) Schott, *Pontederia cordata* L. 'Singapore Pink,' and *Thalia geniculata* f. *rheumoides* (Polomski et al. 2007; 2008). P assimilative capacity of these floating macrophytes could have been affected by the N source and N:P ratio of treatment solutions. Equal amounts of NH_4^+ and NO_3^- and an optimum N:P ratio range of 2.3-5 in water result in maximum biomass yields in water hyacinth (Reddy and Tucker, 1983). Other N and P uptake studies with water hyacinth also suggest that N:P ratio of wastewater affects P removal (Reddy et al., 1989; Reddy et al., 1990; Jayaweera and Kasturiarachchi, 2004); however, this concept was not tested directly.

An analysis of the water that remained in the pots after eight weeks revealed no significant differences between species and treatment levels in the concentration of leftover N and P. Less than 4 and 7% of the original amount of N and P supplied to the plants, respectively, was detected in the remaining solution (data not shown). Of the

original amount of N and P supplied to gravel-only pots, 38 to 48% N and 22 to 58% P remained (data not shown). 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. N depletion may have occurred via denitrification processes. It is unlikely that P precipitation occurred in the gravel-only pots because the pH was not alkaline enough (mean pH of 7.1) to promote precipitation of insoluble tricalcium-phosphate [$Ca_3(PO_4)_2$] complexes (Richardson, 1985). Using 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 confirmed that P precipitation was an unlikely transformation pathway for P removal from the nutrient solution (Gustafsson, 2008).

Nitrogen and Phosphorus Tissue Concentration

Mineral concentrations are typically reported in wetland plant nutrient recovery research, although the contents or the weights of nutrients reflect differences in nutrient accumulation by plants. As expected, the differences in allocation of the nutrients to shoot and roots within species varied by the method in which the results were expressed, i.e., concentration vs. content. N concentration of water hyacinth shoots was greater than roots at the two highest N treatment levels (Table 4.2). A similar trend was observed with P as water hyacinth shoots exhibited a higher sink strength with increasing P treatment levels. Allocation of N and P to above- rather than below-ground water hyacinth parts with increasing N and P levels has been observed by other researchers in free-floating hydroponic experiments (Shiralipour et al. 1981; Reddy and Tucker 1983;

Table 4.2. Mean (n=12) nitrogen (N) and phosphorus (P) concentration and content of shoots and roots of three floating hydrophytes grown for eight weeks in a laboratory-scale subsurface constructed 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. Treatments were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface. Refer to Appendix C for other mineral concentrations.

Treatment level		Concentration				Content			
		N		P		N		P	
N	P	shoot	root	shoot	root	shoot	root	shoot	root
($mg \cdot L^{-1}$)		($mg \cdot g^{-1}$)				(mg)			
Eichhornia crassipes									
0.39	0.07	11.51	10.70	1.54**	1.29	134.400**	19.144	17.954**	2.287
1.75	0.18	10.16	10.19	1.30	1.18	143.480**	22.865	18.804**	2.708
10.44	1.86	11.29	10.38	1.45**	1.26	158.060**	22.749	20.647**	2.763
21.57	3.63	13.042*	11.55	1.78	1.67	284.000**	23.470	38.617**	3.429
36.81	6.77	17.78**	13.08	2.53**	1.71	559.790**	26.784	80.134**	3.502
Myriophyllum aquaticum									
0.39	0.07	8.45	7.39	1.19	1.31	48.538**	15.935	7.304	3.865
1.75	0.18	7.59	7.48	1.23	1.33	43.174**	20.568	6.968	4.775
10.44	1.86	9.28**	7.52	1.45	1.26	84.083**	27.607	13.323**	5.505
21.57	3.63	11.30*	9.28	1.81*	1.41	130.990**	32.313	21.189**	6.211
36.81	6.77	18.15	16.76	2.73*	2.18	257.480**	46.522	38.721**	8.397
Pistia stratiotes									
0.39	0.07	13.93	16.46	2.20	2.66	33.011**	7.472	5.401**	1.097
1.75	0.18	13.19	15.97**	1.88	2.04	34.102**	9.263	5.053**	1.137
10.44	1.86	14.14	16.78**	1.96	2.33*	68.688**	14.718	9.843**	2.039
21.57	3.63	15.75	16.36	2.18	2.22	107.720**	15.392	14.927**	2.111
36.81	6.77	21.08	19.08	3.07**	2.35	217.790**	21.090	31.736**	2.624

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

Xie et al., 2004). Agami and Reddy (1990) also found N concentration of water hyacinth shoots was ~ two-fold higher than roots, but P accumulation was evenly distributed comparable to values reported in dairy lagoon wastewater (DeBusk et al., 1995; Tripathi

and Upadhyay, 2003) and free-floating hydroponic studies (Boyd, 1976; Tucker and DeBusk, 1981).

Parrotfeather shoot N was higher than root N at 10.44 and 1.86 $mg \cdot L^{-1}$ N and P, respectively, and 21.57 and 3.63 $mg \cdot L^{-1}$ N and P, respectively, suggesting N partitioning to shoots with increasing N treatment levels. N concentration of dwarf redstemmed parrotfeather at the highest treatment level was comparable to the N concentration of field-collected parrotfeather sampled from natural stands growing in agricultural drainage canals or from creeks and pools receiving agricultural runoff in central California (Rejmankova, 1992). Phosphorus concentration in parrotfeather was higher in shoots rather than roots at the two highest treatment levels, which indicated an increasing allocation of P to shoots than to roots with increasing P levels.

No N concentration trend was evident with water lettuce, but P concentration indicated a greater allocation of P to shoots with increasing P treatment levels. Shoot P was greater in shoots than roots at 10.44 and 1.86 $mg \bullet L^{-1}$ N and P, respectively, and at the highest treatment level. Our N and P concentrations in water lettuce were similar to other studies (Tucker and DeBusk, 1981; Agami and Reddy 1990) and in CWs (Greenway and Woolley, 1999).

N concentration was greater in water lettuce than water hyacinth (data not presented), which was similar to other studies (Tucker, 1981; Reddy and DeBusk, 1985). We attribute the difference to N dilution caused by water hyacinth's growth rate--among the highest of any plant known (Gopal, 1987); its greater biomass production diluted N assimilated by water hyacinth. Contrary to these findings, Aoi and Hayashi (1996)

reported greater (~ 1.5 times) N and P concentrations in water hyacinth than water lettuce in an outdoor study in Japan involving a continuous flow and batch culture system. Upadhyay et al. (2007) reported "initial" P concentrations of water hyacinth and water lettuce that were 1.3- and 2-fold greater in leaves and roots, respectively, and 1.8- and 3.5-fold higher in water lettuce leaves and roots, respectively, compared to our highest treatment level, but "initial" N concentrations were comparable to ours. The discrepancies in N and P concentration could have resulted from variations in experimental design that includes plant density, temperature, duration of the experiment, solar radiation, and the concentration and ratio of nutrients. The effect of mechanical impedance by the pea gravel substrate on root architecture and nutrient absorption warrants further investigation.

Nitrogen and Phosphorus Tissue Content

Nitrogen content (plant dry weight x tissue N concentration) of the three species was higher in shoots than roots at every treatment level. Water hyacinth allocated ≥ 86% N to shoots compared to roots (Table 4.2). Greatest amount of assimilated N was in water lettuce and parrotfeather shoots (≥ 78% and ≥ 59%, respectively) than roots. This dominant sink strength of shoots at every N treatment level was observed in the marginal aquatic garden plants Louisiana iris hybrid 'Full Eclipse,' *Pontederia cordata* L. 'Singapore Pink,' *Oenenathe javanica* (Blume) DC. 'Flamingo,' *Phyla lanceolata* (Michx.) Greene, *Rhyncospora colorata* (L.) H. Pfeiffer, and *Thalia geniculata* f. *rheumoides* Shuey (Polomski et al., 2007; 2008).

Phosphorus content was also greatest in above-ground organs at every treatment level for the three species. Water hyacinth, water lettuce, and parrotfeather shoots contained ≥ 87, 79, and 59% P, respectively, compared to roots. Also, we observed this partitioning of P to shoots instead of roots with increasing levels of P in *Canna* x *generalis* Bailey (pro sp.) 'Bengal Tiger' and *Colocasia esculenta* (L.) Schott var. *antiquorum* (Schott) Hubbard & Rehd. 'Illustris (Polomski et al., 2007).

The potential application of water hyacinth, parrotfeather, and water lettuce for nutrient attenuation of nursery/greenhouse wastewater must be tempered by their well-documented reputations as noxious weeds in certain regions and ecosystems. Water hyacinth, in particular, possesses a dichotomous nature: one of the world's worst weeds that devastates environmental systems, but demonstrates substantive phytoremediating ability (Holm et al., 1997; Mehra et al., 1999).

Conclusions

Over an eight-week period water hyacinth, water lettuce, and parrotfeather thrived in a gravel-based, laboratory-scale subsurface CW receiving nursery runoff levels of N and P. Nitrogen uptake efficiency was highest in water hyacinth, and N content was greatest in above-ground tissues at every treatment level. Phosphorus recovery rates were similar for the three species and P was preferentially stored in shoots.

Similar to the recommendations of Hadad and Maine (2007), our study supports the possibility of integrating floating aquatic macrophytes with emergent macrophytes in a self-contained polycultural SSF CW system that can be used to remediate runoff from nursery and greenhouse operations. Floating macrophytes may have an important role in

greenhouse production in temperate areas where they can be cultivated indoors in SSF CWs to assimilate NO_3^-, and soluble PO_4^{3-}, and heavy metal trace elements, which are often applied year-round (Biernbaum, 1992). In addition, their ability to process high volumes of nutrient-rich water reduces the amount of effluent that has to be discarded.

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CHAPTER V

EFFECT OF N:P RATIO OF INFLUENT ON BIOMASS, NUTRIENT ALLOCATION AND RECOVERY OF *TYPHA LATIFOLIA* AND *CANNA* 'BENGAL TIGER' IN A LABORATORY-SCALE CONSTRUCTED WETLAND

Abstract

Constructed wetlands (CWs) are an effective low-technology approach for treating agricultural, industrial, and municipal wastewater. Recovery of phosphorus by constructed wetland plants may be affected by wastewater nitrogen to phosphorus (N:P) ratios. Varying N:P ratios were supplied to *Canna* 'Bengal Tiger' and *Typha latifolia* in a laboratory-scale subsurface flow (SSF) CW system with a 4-d hydraulic retention time in a climate-controlled greenhouse. *Typha latifolia* and *Canna* 'Bengal Tiger' received the following five treatments that comprised the following N:P ratios: 6:1, 3:1, 1:1, 1:3 and 1:6. Mean total P concentrations ranged from 6.9 $mg \cdot L^{-1}$ (6:1) to 252.2 $mg \cdot L^{-1}$ P (1:6); nitrate-nitrogen (NO_3-N) was maintained at a constant mean level of 42.4 $mg \cdot L^{-1}$. At 60 d *Canna* shoot P concentration was 13.91 and 19.77 $mg \cdot g^{-1}$ in the 1:3 (126 $mg \cdot L^{-1}$ P) and 1:6 treatments, respectively, which greatly exceeded *Typha* shoot P concentration of 2.4 and 3.0 $mg \cdot g^{-1}$ in the 1:3 and 1:6 treatments. *Typha* and *Canna* whole plant N:P concentration was linearly correlated with N:P treatment ratios. For the 1:3 and 1:6 treatments, *Canna* assimilated 40.7 and 30.6% of supplied P compared to 9.7 and 6.2% for *Typha*. Although both species exhibited luxury consumption of P, *Typha latifolia* may have been nitrogen-limited at the 1:1, 1:3, and 1:6 N:P ratios. Differential accumulation of P relative to N suggests that N:P of wastewater and the N:P assimilation

ability of plants used in constructed treatment wetlands should be considered when designing treatment wetlands for nutrient attenuation.

Introduction

Nitrogen and P are critical nutrients in the life cycles of wetland plants (US EPA, 2000; Kadlec and Knight, 2006), and are traditionally considered in studies of plant mineral nutrition. Both N and P are involved in plant metabolism and growth, and there are numerous points of interaction between N- and P-dependent processes. According to the Sprengel-Liebig Law of the Minimum (Epstein and Bloom, 2005), the most limiting nutrient controls plant growth. Therefore, if plants are deprived of an optimal P supply, the uptake or transport of other nutrients can be altered significantly, which has been well-documented in the literature (e.g., Sutcliffe, 1962; Havlin et al., 2005; Sanchez, 2007).

Chapin et al. (1990) viewed the acquisition and allocation of resources in plants in economic terms where plants attempt to "balance" shortages or excesses to optimize their performance. This homeostatic adjustment of resource concentrations involves physiological and architectural alterations, including root-to-shoot ratio, uptake efficiency of scarce or overabundant resources, and resource allocation patterns (Chapin et al., 1990; Bazzaz, 1997).

Nutrient ratios, termed "nutrient stoichiometry" by Mendez and Karlsson (2005), are used to predict nutrient limitations in ecological sytems. Single elemental ratios, such as N:P, are successfully used to measure nutrient limitations of phytoplankton and

zooplankton communities in natural waters (i.e., Redfield ratio; Redfield, 1958; Ketchum, 1969) and in wetland ecosystems (Koerselman and Meuleman, 1996; Gusewell and Koerselman. 2002), and terrestrial environments (Tessier and Raynal, 2003; Gusewell, 2004) ecosystems. Nutrient ratios may also reflect excess storage of an abundant nutrient rather than the limitation of another (Chapin and Cleve, 1991). An important caveat of N:P ratios to predict nutrient limitation is that it can only be applied to plants that are not limited by other nutrients other than N or P (Koerselman and Meuleman, 1996).

In many constructed wetland studies actual or simulated wastewater effluent is used without regard for the N:P ratio in the effluent (e.g., Cizkova-Koncalova et al., 1996; Romero et al., 1999; Xie et al., 2004; and Kyambadde et al., 2005). Nutrient concentrations in wastewater effluent, mainly N and P, and loading rate vary depending on wastewater quality, wastewater treatment facility type, and season. Changes to nutrient availability may influence plant growth responses and resource allocation, which ultimately affects CW performance and the dynamics of the treatment system (Adler et al., 2008; Zhang et al., 2008a).

Responses to N and P may be specifically determined by absolute N or P supply or by the supply of one relative to the other (Gusewell, 2005). The few studies on the interactive effect of N and P on plant growth are inconsistent among wetland plant species. For example, Ulrich and Burton (1988) found that NO_3-N and P supply and N:P ratios strongly affect growth and biomass of *Typha latifolia* L., *T. angustifolia* L., *Sparganium eurycarpum* Engelm., and *Phragmites australis* (Cav.) Trin. Ex. Steudel.

However, Cary and Weerts (1984) and Romero et al. (1999) did not observe an interactive effect of N and P for either *Typha orientalis* Presl or *P. australis*. Romero (1999) found N supply affects growth of *P. australis*, whereas P did not have any effect; however, an imbalanced supply of N and P suppresses growth of *P. australis*.

Some species appear to be most successful at high N:P supply ratios (e.g., *Molinia caerulea*; Kirkham, 2001; Tomassen et al., 2003), and others at low N:P supply ratios (e.g., *Typha glauca*; Woo and Zedler, 2002). Interestingly, eutrophic species, such as *Typha domingensis* Pers., demonstrate a high degree of flexibility in low P conditions (0.01-0.04 $mg \cdot L^{-1}$), with increased P uptake capacity comparable to a low-nutrient acclimated species *Cladium jamaicense* Crantz (Lorenzen et al., 2001). These studies suggest that species respond differently in terms of biomass production, morphology and/or physiology to the relative supplies of N and P. Knowing how N:P supply ratios affect the growth and uptake of N and P may lead to improved plant selection in vegetated constructed wetlands and enable an understanding and prediction of how changes in relative supplies of N and P in wastewater affect N and P recovery.

The two species evaluated in this study are the monocots *Canna* 'Bengal Tiger,' an upright, rhizomatous herbaceous perennial native to tropical America (Ogden, 2007; Hart Canna, 2009). *Canna* 'Bengal Tiger' has green and yellow-variegated foliage, grows 1.2 to 1.8 m tall, and bears panicles of orange to red-orange flowers (Speichert and Speichert, 2004). *Canna indica* L., an upright perennial rhizomatous herb native to tropical America, and other related species and cultivars, such as *Canna flaccida* and *Canna* 'Red King Humbert,' and Canna 'Yellow King Humbert,' have been used in

constructed wetlands for water quality improvement and landscape restoration due to relatively high nutrient removal efficiency and aesthetic value (Fernandez et al., 1999; Tanner, 2001; Yang et al., 2001; Konnerup et al., 2009; Zhang et al., 2009). *Canna* 'Bengal Tiger' (*Canna*) was included in this study for the following reasons: (a) it flourished in static, waterlogged conditions; (2) it exhibited high rates of biomass accumulation; (3) it recovered N and P near optimal levels in an earlier study (Polomski et al., 2007); (4) its aesthetic features makes it a suitable candidate for production/nutrient attenuation in nurseries and for remediation in commercial and residential landscapes; and (5) *Canna* can be cultivated in aquatic and terrestrial environments, which increases its marketability and utilization.

Broadleaf cattail (*Typha latifolia* L.) was included as a point of comparison because of its widespread use in constructed wetlands for remediation of nutrients, pesticides, and heavy metals (Surrency, 1993; Kvet et al., 1999; Scholz and Lee, 2005; Brisson and Chazarenc, 2009). *Typha latifolia* is a rhizomatous perennial that ranges in height from 1.2–3 m and produces extensive lateral rhizomes, up to 70 cm in length (Holm et al., 1997). *T. latifolia* grows in wet or saturated soils in wet meadows, marshes, along streams and lakes, and in roadside ditches throughout North America, from central Alaska to Mexico (Grace and Harrison, 1986). Although native to North America, the rapid clonal growth of *T. latifolia*, particularly in disturbed, high nutrient environments, has transformed entire wetlands into monotypic cattail stands, classifying *T. latifolia* as a serious aquatic weed (Holm et al., 1997).

Our objective was to investigate differences in N:P ratio (N supply constant) and its effects on growth, nutrient recovery/allocation, and nutrient use efficiency of *Typha latifolia* and *Canna* 'Bengal Tiger.'

Materials and Methods

Study Site

This greenhouse study was conducted from July 13 to September 10, 2008, in Clemson University's Biosystems Research Complex (Clemson, SC, USA; latitude 34°40'8"; longitude 82°50'40"). During the experiment average daily temperatures (°C), relative humidity, and daily light integral (PAR) in the greenhouse were 27.66 °C ± 0.03, 64.01% ± 0.17, and 21.1 ± 0.7 $mol \cdot m^{-2} \cdot d^{-1}$, respectively.

Microcosms

The laboratory-scale microcosm constructed wetland system consisted of 7.6 L white HDPE buckets (24.1-cm height; 20.8-cm bottom diameter, and 24.6-cm top diameter) filled to within 2.5 cm of the top with approximately 52.9 kg washed quartz pea gravel having the following size distribution (% weight): less than 8 mm (33%); 8 to 15 mm (55%), and 15 to 20 mm (12%). The microcosms mimicked subsurface treatment wetlands with the water level maintained at or just beneath the gravel surface (Kadlec and Wallace, 2009). The buckets were placed on gray cinder blocks (20.3-cm x 20.3-cm x 40.6-cm) to allow for drainage via a 13-cm long translucent Tygoprene™ XL-60 pump tubing (12.8 mm OD and 9.6 mm ID) attached to a male adapter nylon fitting (9.5 mm x 9.5 mm [hose ID x male NPT]) secured with 100% silicon and positioned 0.5 cm above

the base of the bucket. A mesh covering the hole was fastened on the inside of the microcosm to prevent loss of gravel during water drainage.

Plant Establishment

Two-inch dormant plugs of *Typha latifolia* (Environmental Concerns, St. Michaels, MD) and individual micropropagated plants of *Canna* 'Bengal Tiger' (AG3, Apopka, FL) were washed free of medium and planted in 16.7-cm diameter 2.8-L aquatic pots filled with sand in Nov and Dec 2007, respectively. All plants were topdressed at a standard rate of 3 $g \cdot pot^{-1}$ of 14N-6.1P-11.6K Osmocote (The Scotts Co., Marysville, OH) and watered as needed. A 16-h photoperiod was maintained during the winter months with 1000 W metal halide lights.

About 40 d prior to the start of the experiment each species was washed free of sand, weighed, and transplanted into individual microcosms. Unplanted gravel systems were used as controls. Each microcosm was filled to the gravel surface with 50 $mg \cdot L^{-1}$ N of 15-2.2-12.5 (Jack's Professional® Water-Soluble Fertilizer, J. R. Peters, Allentown, PA). Every 3 d the buckets were emptied, flushed with tapwater, and replenished with fertilizer solution. Plants were considered ready for experimental use at a growth stage when rhizomes and ramets were produced by the primary shoot system. Three d prior to the start of the experiment, microcosms were flushed and refilled daily with tapwater.

Fertilizer N:P Treatments

Five treatments provided a constant mean level of NO_3-N (42.4 ± 0.6 $mg \cdot L^{-1}$) and the following mean total P concentrations ($mg \cdot L^{-1}$) and N:P ratios: 6:1 (6.9 ± 0.3;

N42:P7); 3:1 (13.7 ± 1.8; N42:P14); 1:1 (41.59 ± 0.4; N42:P42); 1:3 (126.4 ± 0.4; 42N:126P); and 1:6 (252.2 ± 0.6; 42N:252P). The following actual mean concentrations of macro- and micronutrients ($mg \cdot L^{-1}$): K 66.4 ± 0.5; Ca 45.3 ± 0.3; Mg 10.1 ± 0.1; Zn 1.03 ± 0.03; Cu 0.41 ± 0.02; Mn 2.09 ± 0.02; Fe 0.93 ± 0.08; SO_4-S 103.7 ± 7.84; Na 177.75 ± 1.02 ; B 0.34 ± 0; Cl 9.86 ± 0.26. These concentrations approximated a 20% modified Hoagland's nutrient solution no. 1 (Hoagland and Arnon, 1950), which met normal growth requirements of *Canna* 'Bengal Tiger,' *Canna* 'Yellow King Humbert,' *Typha minima*, and several other species in a pea gravel-based laboratory-scale constructed wetland system (Polomski et al., 2007; 2008). The treatments were prepared from commercially available fertilizers and food/laboratory grade chemicals (Table 5.1). Sodium was used as the counter cation in the 1N:1P and higher N:P treatments. Sodium sulfate (Na_2SO_4) was supplied to each treatment to provide constant levels of Na^+ in all treatments.

Treatments were prepared in individual green polyethylene vertical bulk water storage tanks (378.5 L; 162.6-cm height, 58.4-cm diameter (The Tank Depot, Inc., Pompano Beach, FL) with tapwater and continuously agitated with submersible water pumps (ViaAqua™ VA306) to avoid any stratification. Tapwater pH was 6.0 and the electric conductivity was 0.09 $mmhos \cdot cm^{-1}$. Tapwater contained the following nutrient concentrations ($mg \cdot L^{-1}$): 0 NO_3-N, 0.1 PO_4-P, 1.7 K, 3.5 Ca, 1.0 Mg, 3 SO_4-S, 0.01 Zn, 0.03 Cu, 0 Mn, 0.01 Fe, and 0.01 B. After each preparation mineral concentrations of each treatment were determined with a FIALab nitrate analyzer (FIAlab Instruments, Inc., Bellevue, WA) for NO_3-N and the Spectro ARCOS ICP (Spectro, Mahwah, NJ) for

Table 5.1. Fertilizer sources used to produce the five N:P ratio treatments supplement with macro- and micronutrients.

Nutrient	Analysis	Company
Calcium nitrate (15.5-0-0)	YaraLiva™ Calcinit™ greenhouse grade calcium nitrate: 1% NH_4-N; 14.5% NO_3-N; and 19% Ca	Yara North America, Tampa, FL
0-52-34	Multi-MKP® Monopotassium phosphate (0-22.7-28.7)	Haifa Nutritech, Altamonte Springs, FL
K_2SO_4 (0-0-52)	Microsulfate of potash	Harrell's, Lakeland, FL, USA
$MgSO_4$	Magnesium sulfate heptahydrate, technical grade	Harrell's, Lakeland, FL, USA
Na_2SO_4	Anhydrous natural sodium sulfate, industrial use	Baddley Chemicals, Inc., Baton Rouge, LA
NaH_2PO_4	Anhydrous Monosodium phosphate, food grade	Baddley Chemicals, Inc. Baton Rouge, LA
Peters Professional® S.T.E.M. Soluble Trace Element Mix	13.00% S; B 1.35% B; 2.30% Cu; 7.50% Fe; 8.00% Mn; 0.04% Mo; 4.50% Zn.	The Scotts Co., Marysville, OH

macro- and micronutrients. The pH was maintained at 5.8-5.9 with 2N H_2SO_4 or 10 N NaOH.

Each microcosm was manually drained through a tube positioned at the bottom of the container, flushed with tapwater, and then batch-loaded from the top with 2500 mL of treatment solution to maintain the water level at the gravel surface. This 4-d hydraulic residence time (HRT) was acceptable for the removal of N and P species in other constructed wetland studies (Jing et al., 2001; Huett et al., 2005), and was within the 2 to 6 d HRT time of large-scale constructed wetlands (Hunter et al., 2001). Due to extenuating circumstances, this 4-d HRT was not implemented until after two sampling events on Day 1 (initiation) and Day 7, when recorded volumes of treatment solution

were supplied. Between sampling events tapwater was added to replace the water lost to evapotranspiration. Treatments were applied 15 times over the duration of the experiment.

Plant Growth

Canna and *Typha* height was measured at 1, 10, 20, 40, and 60 d after initiation. Height was measured from the top of the container to the highest leaf or tip of the inflorescence. The presence and number of inflorescences were recorded over the 60-d experiment.

Plant Tissue Analysis

Every 20 d plants were harvested to provide sequential plant N and P uptake, partitioning, and recovery data. An initial harvest at treatment initiation was conducted to provide baseline dry weight (reported on a mass and areal basis) and N and P content data.

At each harvest shoots were severed at the gravel surface and roots were hand-washed with a high-pressure water stream and rinsed with distilled water. Plant tissues were weighed and then dried at 80 ^{o}C until a constant weight was obtained. After recording their dry weights, stems, inflorescences, and roots were ground separately in a Wiley mill to pass through a 40-mesh (0.425-mm screen). The wet ash procedure was used for all routine minerals using HNO_3 and H_2O_2. Total plant N concentration was determined with a LECO FP-528 (LECO Corp., St. Joseph, MI). Total plant tissue was prepared for analysis with the wet-ashing procedure and subsequently P concentration

was determined by inductively coupled plasma emission spectrophotometer (Thermo Jarrell Ash TJA 61E, Thermo Fisher Scientific Inc., Waltham, MA). N and P content was determined by multiplying plant part dry weight by nutrient concentration, which normalized differences in nutrient concentrations as a result of growth differences between treatments. Whole plant N and P content was derived from combining above- and below-ground mineral content.

Baseline dry weight (reported on a mass and areal basis) and N and P content data were obtained from both species (n=10) at initiation (d 1). They were handled as previously described to determine initial shoot dry weight, root dry weight, and nutrient concentrations. The baseline data was then subtracted from each harvest value for each treatment giving total dry weight and N and P uptake for each treatment every 20 d.

Effluent Analysis

Every 4 d effluent was drained, collected, and volumes recorded from each microcosm in 2 L HDPE containers and refrigerated at 4º C. Nitrite + nitrate (NO_x-N) and orthophosphate (PO_4-P) effluent samples were filtered through 0.45 uM nylon membrane filters into 1.5-mL IC vials and analyzed with a Dionex AS50 ion chromatograph (IC) with AS50 autosampler (Dionex Corp., Sunnyvale, CA). Samples for total P and non-purgeable organic carbon (NPOC) were collected in 28 mL LDPE Nalgene® and 24 mL glass vials, respectively, preserved with 2 mL 2 N sulfuric acid, and stored at 4 ºC until analysis. Total P concentrations were quantitatively determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Thermo Electron Corp., model IRIS 1000 HR, Franklin, Mass.). Nonpurgeable organic carbon

(NPOC) concentration of effluent collected on 20, 30, 40, and 60 d was analyzed with a Shimadzu TOC-V CPH total organic carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Total P and NPOC contents were calculated by multiplying the concentration of each ion by the total volume of the effluent.

Statistical Analysis

The experiment was conducted as a randomized complete block design initially with 12 replicates of each treatment. Four replicates were removed at each harvest at 20, 40, and 60 d. Data were analyzed using Statistical Analysis Software (SAS Institute Inc., Cary, NC) and all tests used $P \leq 0.05$. Final dry weight and mineral concentration and content data among treatments within and between species were analyzed using analysis of variance. Mean separations were done using least significant difference. Root-to-shoot ratio data were transformed with the square root transformation to obtain normality before analysis. Analysis of variance of unplanted microcosms associated with *Canna* and *Typha* indicated no rep and block effects, but significant treatment effects, so data were pooled. PROC GLIMMIX was used to analyze differences between vegetated and unvegetated microcosms. Linear regressions were performed to determine significance between N:P ratios of whole plant tissues and N:P ratios of treatments.

Results and Discussion

Height and Biomass Production

Typha and *Canna* height and growth index rapidly increased up to d 20 and then slowed thereafter (Appendix; Figure D.1 A and B, Figure D.2). There were no differences between N:P treatments in either species relative to plant height. Increasing P

concentrations (decreasing N:P ratios) did not result in any significant trend among treatments in total plant dry weight accumulation or shoot and root dry weight in *Typha* or *Canna* (Table 5.2). *Typha* shoot:root ratios were constant between treatments at every harvest, but at 60 d the 42N:252P (1:6) treatment resulted in the highest *Canna* shoot:root ratio compared to the other treatments. No differences were observed in biomass accumulation between *Typha* and *Canna* at 20 and 40 d. At 60 d *Typha* root dry weight was 25% greater than *Canna* in the N42:P7 and N42:P14 treatments. These treatments resulted in greater total dry weight in *Typha* than *Canna*. Also, *Typha* total dry weight in the N42:P7 and N42:P14 treatments was 23 and 18% greater than *Canna*. Shoot:root ratio differed between species only at 60 d in the highest P treatment, where *Canna* shoot:root ratio was two-fold greater than *Typha*. The number of inflorescences produced by each species was unaffected by N:P treatments, but *Canna* produced more inflorescences than *Typha* for each treatment at 20 and 40 d (Table 5.2). Biomass accumulation trends were similar when reported on an areal basis (Appendix; Table A.1).

Dry weight partitioning between shoots and roots was highly influenced by P availability in both species (Table 5.2), which was consistent with data from other species where low P conditions favor root production (Lynch et al., 1991; Borch et al., 1998; Hansen and Lynch, 1998; Zhang et al., 2002; Ristvey et al., 2007). Our findings agree with other studies observing shoot dry weight increases and root dry weight decreases with increasing nutrient availability in *C. indica* (Zhang, et al., 2008a), *T. angustifolia* (Steinbachova-Vojtiskova et al., 2006) , and *T. latifolia* (Cizkova-Koncalova et al., 1996). These changes in biomass allocation in response to high N and high P relative to low

Table 5.2. Effect of N:P treatments on average weight data and shoot:root ratio in *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days of treatment (n=4). Means (± SE) within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date.

20 days									
		Typha latifolia				*Canna* 'Bengal Tiger'			
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	Total dry wt. (g)	Shoot[z] dry wt (g)	Root[y] dry wt. (g)	Shoot:root ratio	Total dry wt. (g)	Shoot dry wt (g)	Root dry wt. (g)	Shoot:root ratio
42N/P7	6:1	69.59 (6.46) aA	46.39 (4.02) aA	23.20 (4.25) aA	2.16 (0.30) aA	69.17 (1.30) aA	41.53 (2.31 aA	27.65 (2.26) aA	1.56 (0.23) aA
42N/14P	3:1	73.09 (9.99) aA	47.32 (6.00) aA	25.77 (4.32) aA	1.90 (0.15) aA	79.06 (2.70) bA	47.07 (3.71) abA	31.99 (1.89) abA	1.50 (0.19) aA
42N/42P	1:1	72.83 (10.33) aA	46.42 (3.85) aA	26.42 (6.72) aA	2.08 (0.44) aA	80.30 (1.79) bA	46.96 (2.18) abA	33.34 (0.82) bA	1.41 (0.09) aA
42N/126P	1:3	78.85 (15.16) aA	47.68 (8.95) aA	31.17 (6.22) aA	1.55 (0.04) aA	77.16 (3.67) bA	45.28 (1.76) bA	31.88 (2.21) abA	1.43 (0.06) aA
42N/252P	1:6	72.18 (7.73) aA	45.27 (3.75) aA	26.91 (4.33) aA	1.76 (0.19) aA	84.02 (1.50) bA	52.69 (1.34) bA	31.33 (0.88) abA	1.69 (0.07) aA
40 days									
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	Total dry wt. (g)	Shoot[z] dry wt (g)	Root[y] dry wt. (g)	Shoot:root ratio	Total dry wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)	Shoot:root ratio
42N/P7	6:1	132.50 (9.78) abA	77.47 (4.63) aA	55.03 (8.21) abA	1.51 (0.23) aA	112.89 (2.53) aA	67.42 (0.58) aA	45.47 (2.88) aA	1.50 (0.09) abA
42N/14P	3:1	128.44 (12.32) abA	81.13 (6.70 aA	47.30 (6.39) aA	1.76 (0.14) aA	115.02 (2.20) aA	72.31 (3.48) aA	42.71 (1.33) aA	1.71 (0.14) abA
42N/42P	1:1	130.14 (16.04) abA	75.19 (7.22) aA	54.96 (10.04) abA	1.48 (0.25) aA	116.27 (3.27) aA	66.17 (2.21) aA	50.10 (3.44) aA	1.34 (0.11) aA
42N/126P	1:3	118.22 (12.05) aA	69.85 (5.80) aA	48.37 (6.61) abA	1.50 (0.14) aA	112.68 (1.83) aA	71.23 (1.18) aA	41.45 (1.98) aA	1.73 (0.09) bA
42N/252P	1:6	139.04 (14.38) bA	80.16 (6.13) aA	58.87 (9.69) aA	1.46 (0.22) aA	111.78 (8.71) aA	70.79 (4.50) aA	40.99 (4.75) aA	1.77 (0.14) bA

[z]Includes leaves, stems, and inflorescences (if present).
[y]Includes rhizomes.

Table 5.2. Effect of N:P treatments on average weight data and shoot:root ratio in *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days of treatment (n=4). Means (± SE) within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date (continued).

60 days									
Typha latifolia						*Canna* 'Bengal Tiger'			
Treatment		Total dry wt. (g)	Shoot dry wt (g)	Root[y] dry wt. (g)	Shoot:root ratio	Total dry wt. (g)	Shoot dry wt (g)	Root dry wt. (g)	Shoot:root ratio
N/P ($mg \cdot L^{-1}$)	N:P								
42N/P7	6:1	193.94 (15.16) aB	116.37 (15.90) aA	77.57 (3.30) aB	1.52 (0.24) aA	150.29 (2.94) abA	91.51 (2.10) aA	58.78 (3.37) aA	1.57 (0.11) aA
42N/14P	3:1	176.63 (9.10) aB	99.36 (5.84) aA	77.28 (3.93) aB	1.29 (0.06) aA	145.63 (3.30) aA	87.86 (4.84) aA	57.77 (4.05) aA	1.56 (0.21) aA
42N/42P	1:1	186.47 (11.82) aA	99.41 (3.80) aA	87.06 (8.62) aA	1.17 (0.09) aA	145.77 (7.50) aA	86.53 (5.77) aA	59.23 (3.86) aA	1.48 (0.13) aA
42N/126P	1:3	183.22 (14.14) aA	95.92 (3.13) aA	87.30 (11.06) aA	1.13 (0.09) aA	161.59 (5.49) bA	99.17 (3.54) abA	62.42 (5.86) aA	1.64 (0.17) aA
42N/252P	1:6	180.30 (13.59) aA	104.08 (8.97) aA	76.22 (7.97) aB	1.41 (0.19) aA	160.01 (4.64) abA	110.96 (3.73) bA	49.05 (3.16) aA	2.29 (0.17) bB

[z]Includes leaves, stems, and inflorescences (if present).
[y]Includes rhizomes.

nutrient availability are well-documented (Chapin, 1990; Marschner, 1995; Ericsson, 1995; Lorenzen et al., 2001)

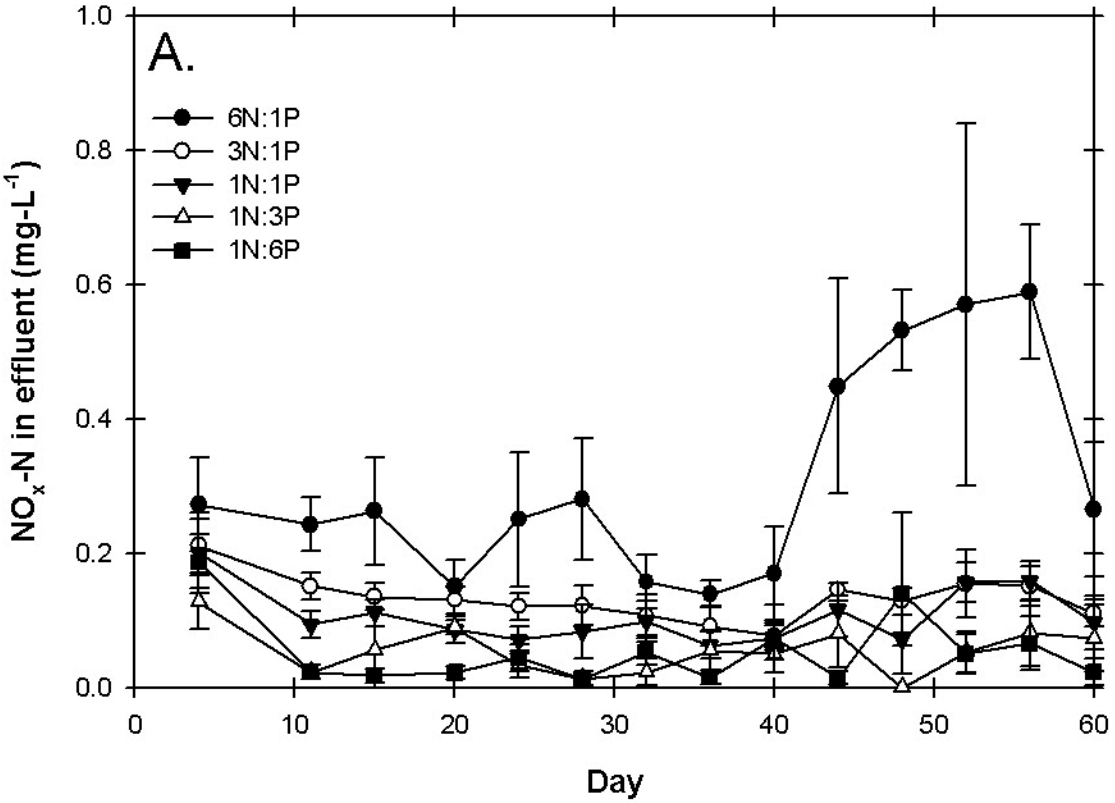
NO_x–N and PO_4–P effluent

No differences in NO_x–N effluent occurred in treatments in either species up to 20 d (Figure 5.1 A and B); subsequently, the N42:P7 treatment in both *Canna* and *Typha* had higher NO_x–N effluent concentrations than the other treatments, especially during the last 20 d of the study. This negligible NO_x–N effluent concentration was markedly below the U.S. Environmental Protection Agency (EPA) maximum allowable contaminant level of 10 $mg \cdot L^{-1}$ NO_3–N in any discharged water (U.S. EPA, 1986). Phosphorus concentration may have been limiting in the N42:P7 treatment, resulting in reduced uptake and transport of NO_3^- from roots to xylem, which precedes large reductions in growth (Lee, 1982; Rufty et al., 1990; Schjorring, 2007).

Unvegetated microcosms had a higher amount of NO_x–N effluent than vegetated microcosms at all sampling dates (Figure 5.2). The higher concentration of NO_x-N remaining in the unplanted systems was consistent with other studies that showed enhance removal of N in vegetated than unvegetated CW systems (Coleman et al., 2001; Yang et al., 2001; Lin et al., 2002; Iamchaturapatr et al., 2007).

In general, *Typha* and *Canna* PO_4–P effluent levels were similar over the duration of the experiment in the N42:P7, N42:P14, and N42:P42 treatments (Figure 5.3 A and B). The two highest P treatments resulted in the highest levels of PO_4–P effluent at every sampling event in both species. *Typha* generally had higher PO_4–P effluent levels than *Canna* in the N42:P42, 42N:126P, and 42N:252P treatments. Orthophosphate effluent

Figure 5.1. Incremental NO_x-N ($mg \cdot L^{-1}$) effluent (July-August) of broadleaf cattail (*Typha latifolia*) and *Canna* 'Bengal Tiger' (A and B, respectively). Vertical bars = ± SE. Each point is the mean of n=12 (d 4-20), n=8 (d 24-40), and n=4 (d 44-60).

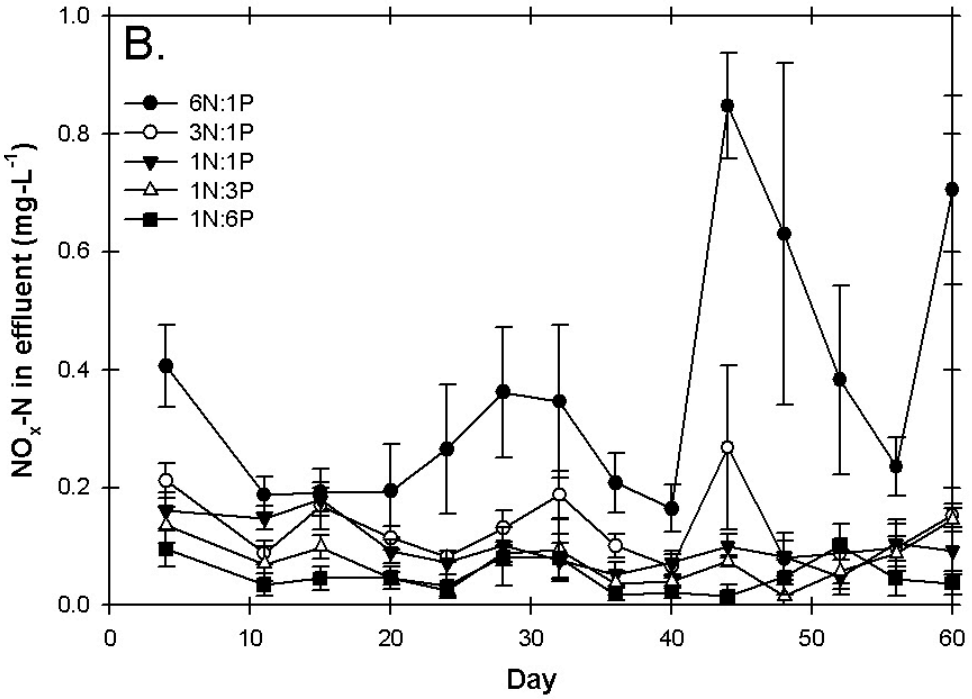


N:P	N/P ($mg \cdot L^{-1}$)	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	bA[z]	cA	cA	bA	bA	bA	cA	cA	aA	bA	bA	bA	bB	bA
3:1	42/14	abA	bB	bA	bA	aA	aA	bcA	bcA	aA	aA	aA	aA	aA	aA
1:1	42/42	abA	bA	abA	abA	aA	aA	bcA	abA	aA	aA	aA	aA	aA	aA
1:3	42/126	aA	aA	abA	bB	aA	aA	aA	abA	aA	aA	aA	aA	aA	aA
1:6	42/252	abA	aA	aA	aA	aA	aA	abA	aA	aA	aA	aA	aA	aA	aA

[z]Means followed by different lowercase letter are significantly different within species at each sampling date according to Least Significant Difference test ($P \leq 0.05$). Uppercase letters indicate significant differences between treatment means of species at each sampling event.

levels in *Canna* were > 79 $mg \cdot L^{-1}$ and > 178 $mg \cdot L^{-1}$ for the 42N:126P and 42N:252P treatments, respectively, compared to *Typha* whose PO_4–P effluent levels exceeded

Figure 5.1. Incremental NO_x-N ($mg \cdot L^{-1}$) effluent (July-August) of broadleaf cattail (*Typha latifolia*) and *Canna* 'Bengal Tiger' (A and B, respectively). Vertical bars = ± SE. Each point is the mean of n=12 (d 4-20), n=8 (d 24-40), and n=4 (d 44-60) (continued).

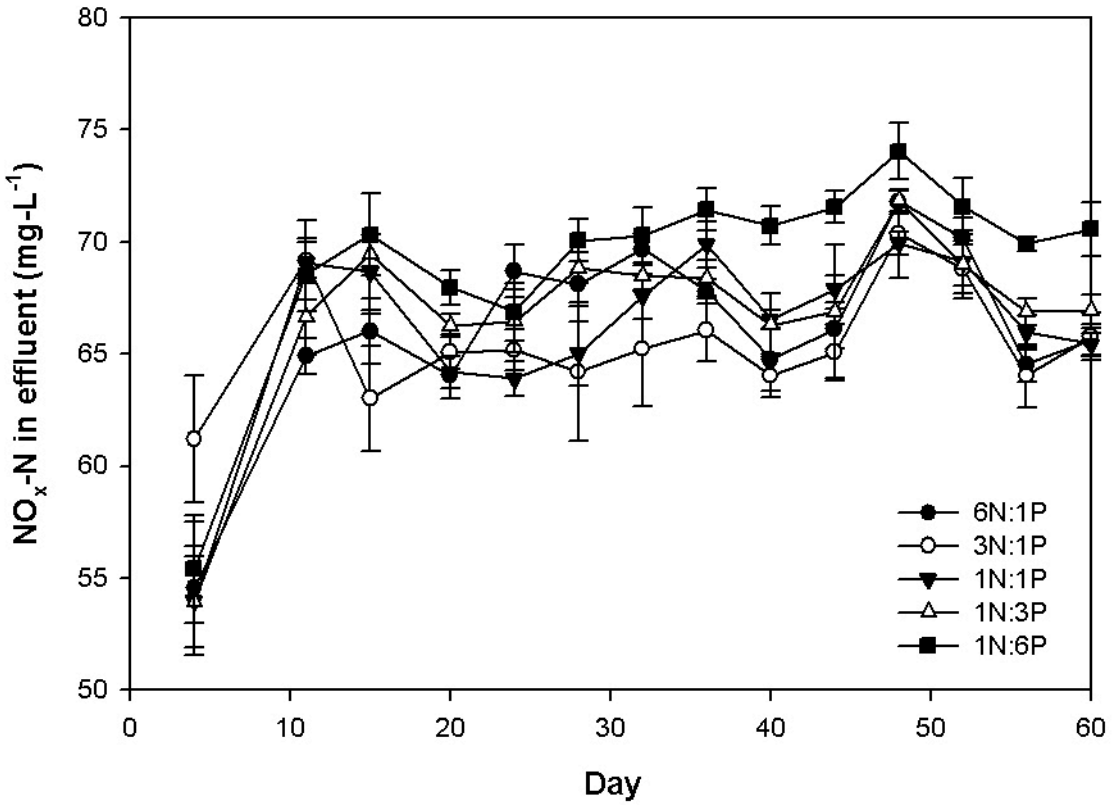


N:P	N/P ($mg \cdot L^{-1}$)	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	bA[z]	cA	cA	bA	bA	bA	bA	cA	bA	cA	bA	bA	bA	bA
3:1	42/14	aA	abA	bcA	abA	aA	aA	abB	bA	aA	bA	aA	aA	aA	aA
1:1	42/42	aA	bcB	cB	abA	aA	aA	aA	abA	aA	abA	aA	aA	aA	aA
1:3	42/126	aA	aA	abA	aA	aA	aB	aA	abA	aA	abA	aA	aA	aA	aA
1:6	42/252	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA

[z]Means followed by different lowercase letter are significantly different within species at each sampling date according to Least Significant Difference test ($P \leq 0.05$). Uppercase letters indicate significant differences between treatment means of species at each sampling event.

106 $mg \cdot L^{-1}$ and 219 $mg \cdot L^{-1}$ for 42N:126P and 42N:252P treatments, respectively. Highest effluent PO_4–P levels of both species occurred at 24-36 d in the 42N:126P and 42N:252P treatments. Orthophosphate levels were highest in *Typha* with PO_4–P levels in the

Figure 5.2. Incremental NO_x-N (mg L^{-1}) effluent (July-August) in unplanted gravel-filled microcosms. Vertical bars = ± SE. Each point is the mean of n=8 containers. Note: NO_x-N concentrations were significantly higher in unplanted microcosms at each treatment at each sampling event compared to the planted microcosms.



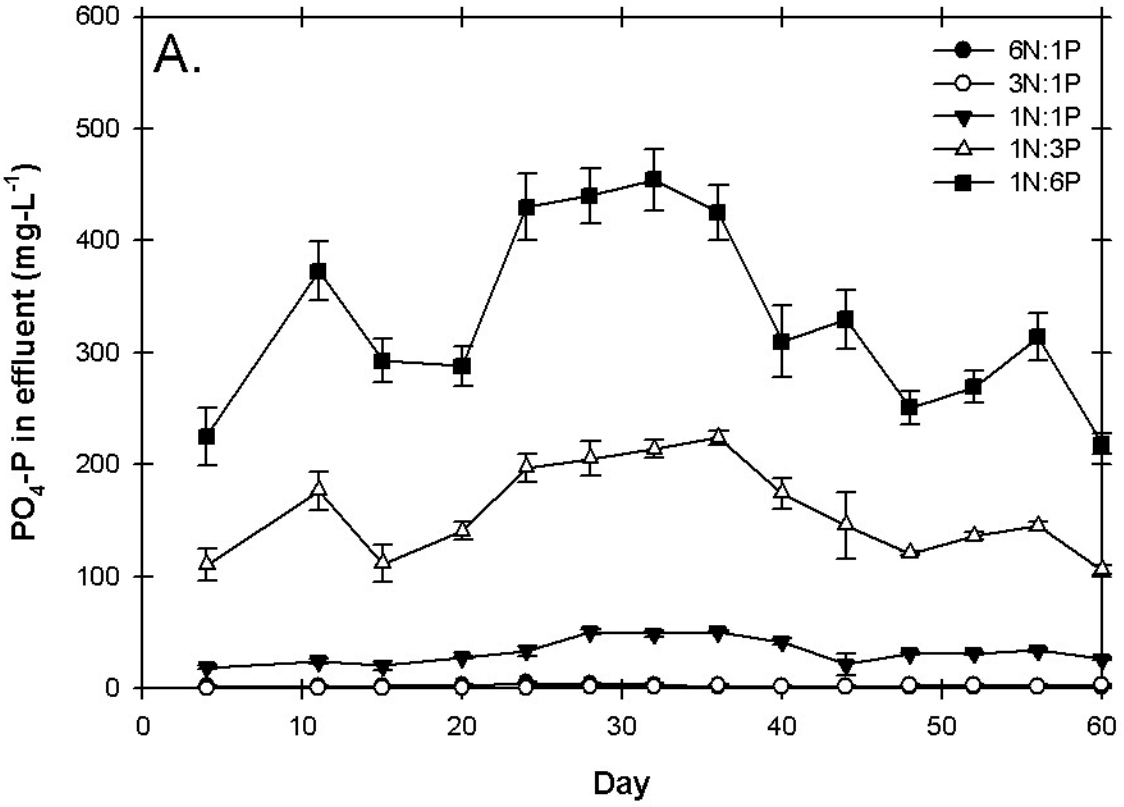
N:P	N/P (mg·L^{-1})	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	a[z]	a	ab	a	b	bc	b	ab	a	a	ab	a	a	a
3:1	42/14	b	b	a	a	a	a	a	a	a	a	a	a	a	a
1:1	42/42	a	b	ab	a	a	ab	ab	bc	a	ab	a	a	ab	a
1:3	42/126	a	ab	b	ab	a	bc	ab	ab	a	a	ab	a	b	a
1:6	42/252	b	ab	b	b	ab	c	b	c	b	b	b	a	c	b

[z]Means followed by different lowercase letter are significantly different at each sampling date according to Least Significant Difference test ($P \leq 0.05$).

42N:252P treatment that were 45 and 57% higher than *Canna* at 28 and 32 d, respectively.

Orthophosphate effluent concentration was highest in the unplanted microcosms

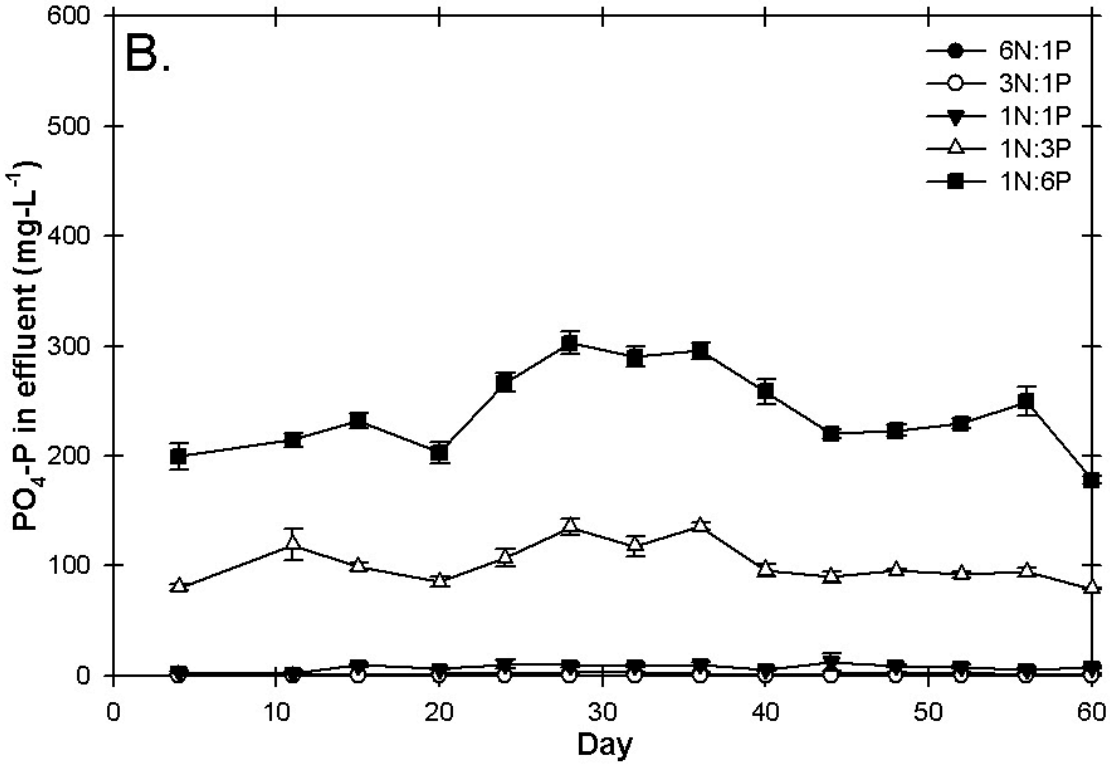
Figure 5.3. Incremental PO_4-P ($mg \cdot L^{-1}$) effluent (July-August) for *Typha latifolia* and *Canna* 'Bengal Tiger' (A and B, respectively). Vertical bars = ± SE. Each point is the mean of n=12 (d 4-20), n=8 (d 24-40), and n=4 (d 44-60).



N:P	N/P ($mg \cdot L^{-1}$)	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	aA[z]	aA	aB	aA	aB	aA	aA	aA	aA	aA	aA	aA	aA	aA
3:1	42/14	aA	aB	aB	aB	aB	aA	aB	aB	aA	aA	aA	aA	aA	aB
1:1	42/42	aA	aB	aB	bB	aB	bB	bB	bB	aB	aA	bB	bA	bB	bB
1:3	42/126	bA	bB	bA	cB	bB	cB	cB	cB	bB	bA	cB	cB	cB	cB
1:6	42/252	cA	cB	cB	dB	cB	dB	dB	dB	cA	cB	dA	dA	dB	dB

[z]Means followed by different lowercase letter are significantly different within species at each sampling date according to LSD test ($P \leq 0.05$). Uppercase letters indicate significant differences between treatment means of species at each sampling event.

Figure 5.3. Incremental PO_4-P ($mg \cdot L^{-1}$) effluent (July-August) for *Typha latifolia* and *Canna* 'Bengal Tiger' (A and B, respectively). Vertical bars = ± SE. Each point is the mean of n=12 (d 4-20), n=8 (d 24-40), and n=4 (d 44-60) (continued).



N:P	N/P ($mg \cdot L^{-1}$)	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	aA[z]	aA	abA	aA	aA	aA	aA	abA	aA	aA	abA	abA	aA	aA
3:1	42/14	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA	aA
1:1	42/42	aA	aA	bA	aA	aA	aA	aA	bA	aA	aA	bA	bA	aA	bA
1:3	42/126	bA	bA	cA	bA	bA	bA	bA	cA	bA	bA	cA	cA	bA	cA
1:6	42/252	cA	cA	dA	cA	cA	cA	cA	dA	cA	cA	dA	dA	cA	dA

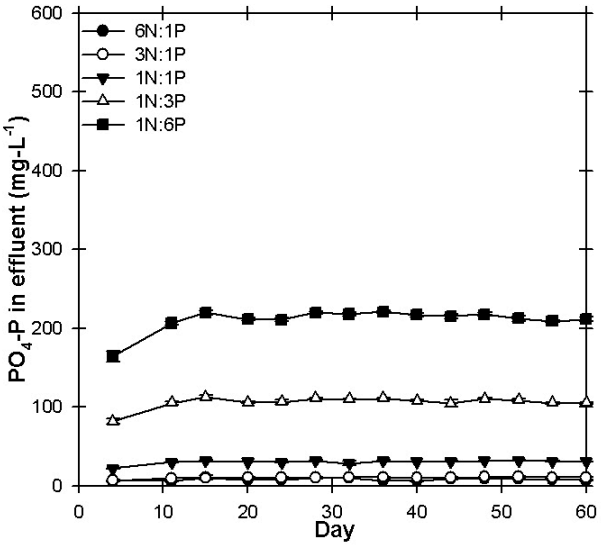
[z]Means followed by different lowercase letter are significantly different within species at each sampling date according to LSD test ($P \leq 0.05$). Uppercase letters indicate significant differences between treatment means of species at each sampling event.

compared to the plant microcosms for the N42:P7 and N42:P14 treatments (Fig. 5.4). Orthophosphate effluent concentrations of unplanted microcosms in the N42:P42 treatment were higher than *Canna* at every sampling event, but comparable or higher than

Typha, particularly from 28-40 d. (Figure 5.4). In general, PO_4–P effluent concentrations in the 42N:126P and 42N:252P treatments were similar in gravel-only and *Canna* microcosms. The high concentration of PO_4–P effluent in the *Typha* microcosms resulted from water losses due to evapotranspiration, which increased the concentration of PO_4–P ($mg \cdot L^{-1}$) remaining in solution that was not absorbed by *Typha*. From a mass balance approach, the content of PO_4–P (mg) that remained was consistently less than the amount of PO_4–P supplied to each species for each treatment and at every sampling event (Appendix D, Figure D.3.).

Rhizodeposition may have contributed to the addition of P in effluent. NPOC levels were higher in vegetated microcosms than in gravel alone, and NPOC levels were highest in *Canna* compared to *Typha* at 30 and 60 d in all treatments (Figure 5.5). Root exudates contribute to nitrification and microbial activities and include carbohydrates, sugars, amino acids, enzymes, vitamins such as thiamine, riboflavin, and pyridoxine, etc., antibiotics, organic acids such as malate, citrate, amino acids, benzoic acids, and phenol and other organic compounds (Rovira, 1969; Brix, 1997; Stottmeister et al., 2003; Bai et al., 2004). *Canna indica* exudes phosphatase in the presence of triazophos [(O, O-diethyl-O-(1-phenyl-1, 2, 4-triazole-3-base) sulfur phosphate] and in the absence of inorganic P (Cheng et al., 2007). Current knowledge of composition of root exudates of helophytes is very limited and further investigation is required to determine how root exudates influence microbial activity and nutrient uptake and transformation.

Figure 5.4. Incremental PO_4-P (mg L^{-1}) effluent (July-August) in unplanted gravel-filled microcosms. Vertical bars = ± SE. Each point is the mean of n=8 containers.



Unplanted

N:P	N/P (mg·L^{-1})	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	aB	aB	aB	aB	aC	aB	aB	aB	aB	aB	aB	aB	aB	aB
3:1	42/14	aC	aB	aB	bC	aC	aC	aC	bC	bC	aB	aC	aC	bC	aC
1:1	42/42	bB	bB	bC	cC	bB	bB	bB	cB	cB	bA	bB	bB	cB	bC
1:3	42/126	cA	cA	cA	dB	cA	cA	cA	dA	dA	cAB	cB	cB	dB	cB
1:6	42/252	dA	dA	dA	eA	dA	dA	dA	eA	eA	dA	dA	dA	eA	dB

Typha latifolia

N:P	N/P (mg·L-1)	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	A	A	A	A	B	A	A	A	A	A	A	A	A	A
3:1	42/14	A	A	A	B	B	B	B	B	B	A	B	B	B	B
1:1	42/42	B	B	B	B	B	C	C	C	C	A	B	B	B	B
1:3	42/126	B	B	A	C	B	C	B	C	B	B	C	C	C	B
1:6	42/252	B	B	B	B	C	C	C	C	B	B	B	B	C	B

Canna 'Bengal Tiger'

N:P	N/P (mg·L-1)	4	11	15	20	24	28	32	36	40	44	48	52	56	60
6:1	42/7	A	A	A	A	A	A	A	A	A	A	A	A	A	A
3:1	42/14	A	A	A	A	A	A	A	A	A	A	A	A	A	A
1:1	42/42	A	A	A	A	A	A	A	A	A	A	A	A	A	A
1:3	42/126	A	A	A	A	A	B	A	B	A	A	A	A	A	A
1:6	42/252	AB	A	A	A	B	B	B	B	AB	A	A	A	B	A

[z]Means followed by different lowercase letter are significantly different within species at each sampling date according to LSD test ($P \leq 0.05$). Uppercase letters indicate significant differences between treatment means of species at each sampling event.

Figure 5.5. Effect of N:P treatments on nonpurgeable organic carbon (NPOC) in *Typha latifolia* (A) and *Canna* 'Bengal Tiger' (B) at 20, 40 and 60 d (n=4) in a greenhouse experiment conducted from July-August 2008. Vertical bars = ± SE. Unplanted gravel microcosms were included as controls.

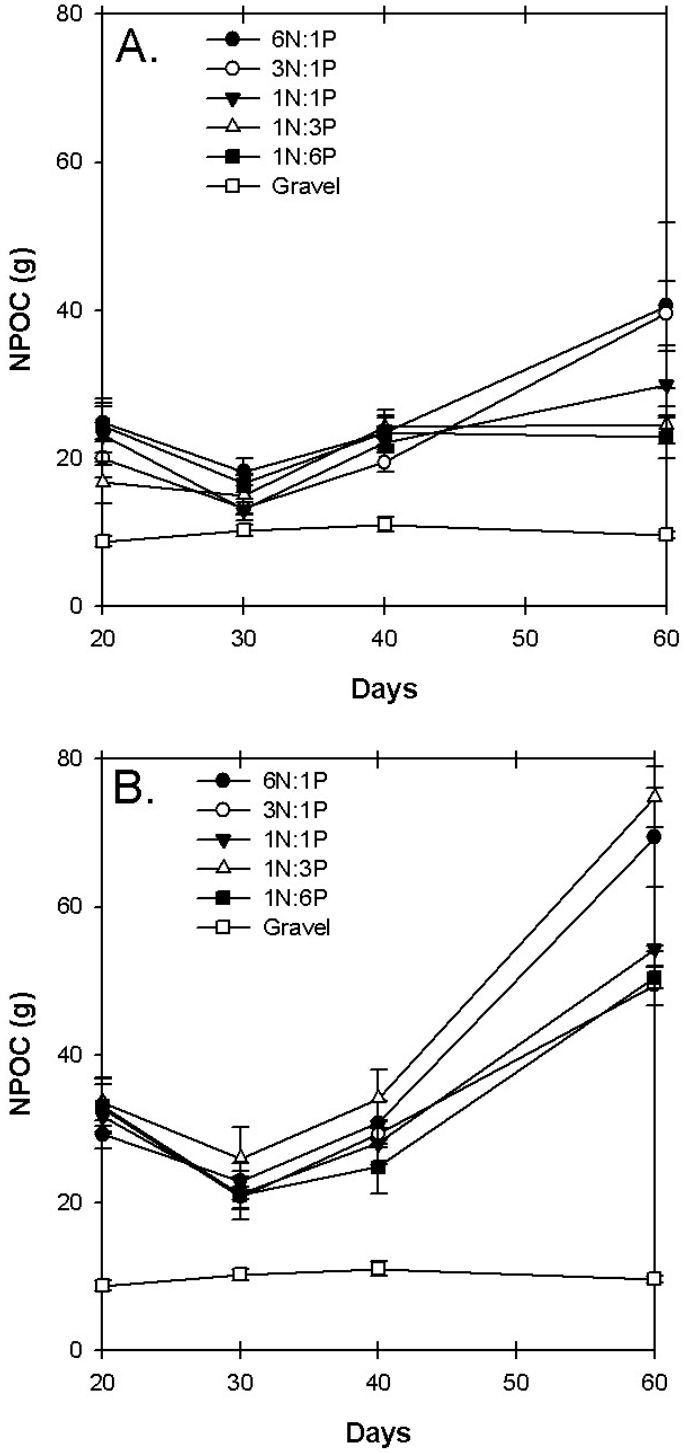


Figure 5.5. Effect of N:P treatments on nonpurgeable organic carbon (NPOC) in *Typha latifolia* (A) and *Canna* 'Bengal Tiger' (B) at 20, 40 and 60 d (n=4) in a greenhouse experiment conducted from July-August 2008. Unplanted gravel microcosms were included as controls (continued).

Typha latifolia					
Treatment					
N/P ($mg \cdot L^{-1}$)	N:P	20 d	30 d	40 d	60 d
42N/P7	6:1	bB	aA	aB	aB
42N/14P	3:1	ab	aA	aB	aB
42N/42P	1:1	bAB	aA	aB	aB
42N/126P	1:3	aB	aA	aB	aB
42N/252P	1:6	bB	aAB	aB	aB
Canna 'Bengal Tiger'					
42N/P7	6:1	aB	abB	abC	bcC
42N/14P	3:1	aC	aB	abC	aB
42N/42P	1:1	aB	abB	abC	abC
42N/126P	1:3	aC	bB	bC	cC
42N/252P	1:6	aB	abB	aB	aC
Unplanted gravel microcosms					
42N/P7	6:1	aA	aA	aA	bA
42N/14P	3:1	aA	aA	aA	abA
42N/42P	1:1	aA	abA	aA	aA
42N/126P	1:3	aA	abA	aA	aA
42N/252P	1:6	aA	bA	aA	abA

[z]Means followed by different lowercase letter are significantly different within species at each sampling date according to LSD test ($P \leq 0.05$). Uppercase letters indicate significant differences between treatment means of species at each sampling event.

Nitrogen and Phosphorus Recovery In Plant Tissues

Mineral concentrations are typically reported in wetland plant nutrient recovery research, although the contents (plant dry weight x tissue N or P concentration) of nutrients reveal differences in nutrient accumulation by plants. In general, there were no trends in *Canna* and *Typha* shoot and root N concentration or content between treatments

at 20, 40, or 60 d (Table 5.3). Between species *Canna* and *Typha* shoot N concentration were similar at 20 and 40 d in each treatment, but at 60 d *Canna* shoot N concentration was higher than cattail in the four highest P treatments. *Canna* root N concentration was highest at every treatment at 40 and 60 d. Both species allocated > 64% N in shoots at every harvest and every treatment. Our results were consistent with Ulrich and Burton (1988) who found that aboveground N concentration of *Typha latifolia* was higher than belowground when N was limiting growth. In our study N became limiting with increases in P, thereby resulting in greater partitioning of N to shoots than roots. Contrary to our study, N concentration increased in heterotrophic structures instead of aboveground tissues with increasing nutrient availability in roots and rhizomes of *T. angustifolia* and rhizomes of *T. latifolia* (Cizkova-Koncalova et al., 1996). However, N probably was not limiting in those studies.

Phosphorus accumulation increased with P availability in both species with concentration and content following identical trends in *Canna* and *Typha*. Canna shoot P concentration was greater than roots with increasing P concentration and content in shoots and roots with increasing P supply concentration (Table 5.4). *Canna* allocated 65% of P uptake to shoots in all treatments, similar to an earlier study with *Canna* 'Bengal Tiger' (Polomski et al., 2007), and *Canna indica* allocated > 56% to shoots at all low, medium, and high N:P ratios (Zhang et al., 2008a).

Typha followed a trend of increasing P concentration and content with increasing levels of P, shoot P concentration was highest at 42N:252P than other treatments at 40 and 60 d, and root P concentration was highest in 42N:252P at 60 d. Shoot P content was

Table 5.3. Effect of N:P treatments on total nitrogen concentration and content (concentration x dry weight) of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days of treatment (n=4). Means (± SE) within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date.

20 days										
		Typha latifolia					*Canna* 'Bengal Tiger'			
Treatment										
N/P ($mg \cdot L^{-1}$)	N:P	Shoot N ($mg \cdot g^{-1}$)	Shoot N (mg)	Root N ($mg \cdot g^{-1}$)	Root N (g)		Shoot N ($mg \cdot g^{-1}$)	Shoot N (mg)	Root N ($mg \cdot g^{-1}$)	Root N (g)
42N/P7	6:1	15.52 (0.40) bA	715.33 (43.61) aA	8.18 (0.19) aA	189.23 (33.19) aA		16.40 (0.98) aA	680.34 (49.54) aA	12.95 (0.70) bB	354.48 (22.02) aB
42N/14P	3:1	16.30 (1.25) bA	681.81 (54.78) aA	9.00 (0.60) aA	226.82 (30.17) aA		17.02 (0.94) aA	773.98 (50.09) bcA	11.57 (0.26 aA	381.29 (28.25) aB
42N/42P	1:1	15.11 (0.68) bA	695.48 (38.26) aA	8.65 (0.80) aA	214.49 (40.08) aA		16.11 (0.78) aA	751.43 (4.18) abcA	11.45 (0.43) aA	381.82 9 (17.80) aB
42N/126P	1:3	12.62 (1.26) aA	699.64 (64.85) aA	8.60 (0.93) aA	251.05 (33.31) aA		15.53 (0.22) aA	702.54 (25.15) abA	12.13 (0.19) abB	386.76 (28.16) aB
42N/252P	1:6	14.56 (0.64) abA	652.55 (32.49) aA	8.03 (0.42) aA	213.82 (32.92) aA		15.72 (0.40) aA	826.41 (5.89) cB	11.43 (0.39) aB	358.67 (20.40) aB
40 days										
Treatment										
N/P ($mg \cdot L^{-1}$)	N:P	Shoot N ($mg \cdot g^{-1}$)	Shoot N (mg)	Root N ($mg \cdot g^{-1}$)	Root N (g)		Shoot N ($mg \cdot g^{-1}$)	Shoot N (mg)	Root N ($mg \cdot g^{-1}$)	Root N (g)
42N/P7	6:1	12.57 (1.01) aA	924.79 (83.95) aA	7.35 (0.41) aA	395.71 (42.66) abA		15.52 (0.84) aA	1046.55 (60.89) aA	11.80 (0.40) aB	534.41 (26.05) aB
42N/14P	3:1	12.87 (0.74) aA	1036.26 (82.84) aA	7.45 (0.34) aA	349.69 (44.94) aA		14.83 (0.70) aA	1070.17 (60.23) aA	11.60 (0.23) aB	495.04 (14.27) aB
42N/42P	1:1	12.75 (1.11) aA	944.39 (77.36) aA	7.13 (0.41) ±aA	380.30 (52.86) abA		14.98 (0.66) aA	987.39 (21.71) aA	11.30 (0.60) aB	561.14 (20.43) aB
42N/126P	1:3	12.83 (0.58) aA	889.67 (62.04) aA	8.05 (0.66) aA	376.36 (29.52) abA		15.06 (0.28) aA	1072.28 (23.22) aA	11.65 (0.28 aB	482.57 (23.02) aB
42N/252P	1:6	12.57 (0.50) aA	936.90 (47.35) aA	7.60 (0.34) aA	438.13 (58.32) bA		15.98 (1.03) aA	1122.06 (53.66) aB	12.18 (0.55) aB	491.54 (36.34) aA

[z]Includes leaves, stems, and inflorescences (if present).
[y]Includes rhizomes.

Table 5.3. Effect of N:P treatments on total nitrogen concentration and content (concentration x dry weight) of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days of treatment (n=4). Means (± SE) within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date (continued).

60 days									
		Typha latifolia				*Canna* 'Bengal Tiger'			
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	Shoot N ($mg \cdot g^{-1}$)	Shoot N (mg)	Root N ($mg \cdot g^{-1}$)	Root N (g)	Shoot N ($mg \cdot g^{-1}$)	Shoot N (mg)	Root N ($mg \cdot g^{-1}$)	Root N (g)
42N/P7	6:1	11.15 (0.03) aA	1287.03 (151.44) bA	6.70 (0.37) aA	517.81 (23.61) aA	13.54 (0.49) abA	1239.09 (60.95) aA	11.73 (0.27) aB	689.79 (53.65) aA
42N/14P	3:1	11.39 (0.29) aA	1127.97 (47.03) abA	7.08 (0.36) aA	543.66 (18.67) aA	14.82 (0.44) bcB	1301.78 (83.09) aA	11.50 (0.27) aB	661.76 (37.32) aB
42N/42P	1:1	11.15 (0.45) aA	1104.65 (32.22) abA	6.53 (0.15) aA	566.56 (54.44) aA	15.02 (0.25) cB	1299.36 (91.24) aA	11.65 (0.39) aB	687.48 (37.45) aA
42N/126P	1:3	10.83 (0.14) aA	1037.49 (26.37) aA	6.70 (0.41) aA	580.83 (68.42) aA	13.42 (0.29) aB	1333.81 (71.70) aB	11.28 (0.36) aB	703.60 (69.04) aA
42N/252P	1:6	10.77 (0.41) aA	1112.89 (71.06) abA	7.00 (0.84) aA	534.98 (87.36) aA	14.68 (0.37) abcB	1580.48 (27.29) bB	11.43 (0.48) aB	555.30 (34.36) aA

[z]Includes leaves, stems, and inflorescences (if present).
[y]Includes rhizomes.

highest at 40 and 60 d for 42N:252P treatment. *Typha* root P content increased with decreasing N:P ratios at every harvest date. *Typha* root P content was similar between N42:P42, 42N:126P, and 42N:252P treatments at 20 and 60 d. At d 40 root content was highest at 42N:252P compared to other treatments. *Typha* shoot sink strength declined at every harvest date across all treatments with > 52%, > 47%, and > 42% P allocated to shoots at 20, 40, and 60 d respectively. This storage of excess P in belowground structures was also found by Ulrich and Burton (1988) with *Typha latifolia*. The higher level of P accumulation in *Typha* roots than shoots could contribute to P discharge into solution via decomposition and rhizodeposition, and also has consequences on the practice of harvesting as a mechanism for P-removal from the system.

Canna shoot P concentration exceeded shoot P concentration of *Typha* for every treatment at 40 and 60 d. *Canna* root P concentration was higher than *Typha* root P concentration at 40 and 60 d for the N42:P42, 42N:126P, and 42N:252P treatments. *Canna* shoot P content followed a similar trend as concentration, with higher *Canna* shoot P content in nearly every treatment and nearly at every harvest. Root P concentration was greater than *Typha* at 40 and 60 d in the N42:P42, 42N:126P, and 42N:252P treatments. Root P content showed no evident trend in any of the *Canna* and *Typha* treatments. Shoot N concentrations of *Canna* were lower than the 28.7 $mg \cdot g^{-1}$ N concentration of "five mature leaves from new growth" of a "hybrid canna lily (*Canna* x *generalis* [*sic*])" reported by Mills and Jones (1996), and lower than the leaf N and shoot N concentrations of *Canna* 'Red King Humbert' (35.4 and 19.0 $mg \cdot g^{-1}$, respectively), which received 23 $mg \cdot L^{-1}$ N (NH_4-N:NO_3-N =14:1) and 6 $mg \cdot L^{-1}$ P

Table 5.4. Effect of N:P treatments on total phosphorus concentration and content (concentration x dry weight) and whole plant N:P concentration of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days of treatment (n=4). Means (± SE) within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date.

20 days											
		Typha latifolia					*Canna* 'Bengal Tiger'				
Treatment											
N/P ($mg \cdot L^{-1}$)	N:P	Shoot P ($mg \cdot g^{-1}$)	Shoot P (mg)	Root P ($mg \cdot g^{-1}$)	Root P (g)	Whole Plant N:P	Shoot P ($mg \cdot g^{-1}$)	Shoot P (mg)	Root P ($mg \cdot g^{-1}$)	Root P (g)	Whole Plant N:P
42N/P7	6:1	1.83 (0.12) aA	84.13 (5.73) aA	2.08 (0.14) aA	46.75 (6.52) aA	6.89 (0.21) dA	2.44 (0.13) aA	101.34 (7.14) aA	1.93 (0.11) aA	52.89 (4.33) aA	6.70 (0.14) eA
42N/14P	3:1	2.38 (0.26) abA	107.97 (3.93) aA	2.85 (0.29) aA	70.05 (6.81) abA	5.11 (0.21) cA	2.97 (0.10) aA	138.80 (7.62) aB	2.30 (0.09) aA	73.63 (5.35) bA	5.54 (0.15) dA
42N/42P	1:1	3.02 (0.21) bcA	137.94 (5.57) bA	3.93 (0.31) bA	97.50 (18.73) bcA	3.88 (0.09) bB	5.62 (0.23) bB	262.61 (5.29) bB	3.85 (0.12) bA	128.37 9 (5.15) cA	2.90 (0.03) cA
42N/126P	1:3	3.32 (0.63) cA	142.83 (15.28) bcA	4.55 (0.49) bcA	132.87 (17.92) cA	3.28 (0.10) aB	10.12 (0.50) cB	457.49 (23.82) cB	5.95 (0.18) cB	188.47 (7.14) dA	1.69 (0.05) bA
42N/252P	1:6	3.75 (0.18) cA	171.36 (21.57) cA	4.88 (0.27) cA	131.74 (24.52) cA	2.95 (0.22) aB	13.28 (0.40) dB	700.84 (34.68) dB	8.35 (0.46) dB	260.57 (9.54) eB	1.24 (0.07) aA
40 days											
Treatment											
N/P ($mg \cdot L^{-1}$)	N:P	Shoot P ($mg \cdot g^{-1}$)	Shoot P (mg)	Root P ($mg \cdot g^{-1}$)	Root P (g)	Whole Plant N:P	Shoot P ($mg \cdot g^{-1}$)	Shoot P (mg)	Root P ($mg \cdot g^{-1}$)	Root P (g)	Whole Plant N:P
42N/P7	6:1	1.43 (0.09) aA	109.93 (7.51) aA	1.98 (0.28) aA	103.14 (8.21) aA	6.13 (0.25) dA	2.41 (0.16) aB	162.21 (7.03) aB	1.80 (0.07) aA	81.60 (4.67) aA	6.51 (0.36) dA
42N/14P	3:1	2.05 (0.13) bA	163.96 (6.47) bA	2.85 (0.20) bA	131.25 (9.80) aA	4.69 (0.23) cA	3.27 (0.11) bB	236.30 (10.60) aB	2.55 (0.10) aA	109.13 (6.59) aA	4.53 (0.10) cA
42N/42P	1:1	2.30 (0.14) bcA	172.19 (16.17) bA	3.65 (0.20) cA	194.82 (26.80) bA	3.65 (0.21) bB	7.55 (0.35) cB	498.03 (14.74) bB	4.88 (0.12) bB	243.75 (14.97) bA	2.09 (0.03) bA
42N/126P	1:3	2.63 (0.09) cA	182.07 (11.44) bA	4.13 (0.14) cdA	200.70 (29.83) bA	3.36 (0.20) abB	13.80 (0.19) dB	968.67 (21.43) cB	8.53 (0.23) cB	353.56 (20.36) cB	1.18 (0.03) aA
42N/252P	1:6	3.05 (0.17) dA	246.37 (29.86) cA	4.75 (0.30) dA	275.71 (43.39) cA	2.90 (0.16) aB	18.98 (0.40) eB	1344.38 (93.07) dB	11.88 (0.52) dB	481.88 (45.86) dA	0.89 (0.04) aA

[z]Includes leaves, stems, and inflorescences (if present).
[y]Includes rhizomes.

Table 5.4. Effect of N:P treatments on total phosphorus concentration and content (concentration x dry weight) and whole plant N:P concentration of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days of treatment (n=4). Means (± SE) within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date (continued).

60 days												
		Typha latifolia						*Canna* 'Bengal Tiger				
Treatment												
N/P ($mg \cdot L^{-1}$)	N:P	Shoot P ($mg \cdot g^{-1}$)	Shoot P (mg)	Root P ($mg \cdot g^{-1}$)	Root P (g)	Whole Plant N:P		Shoot P ($mg \cdot g^{-1}$)	Shoot P (mg)	Root P ($mg \cdot g^{-1}$)	Root P (g)	Whole Plant N:P
42N/P7	6:1	1.44 (0.03) aA	145.09 (8.31) aA	1.67 (0.09) aA	131.55 (4.42) aB	6.10 (0.05) eA		2.23 (0.16) aB	203.57 (13.83) aA	1.60 (0.06) aA	94.42 (8.11) aA	6.49 (0.17) dA
42N/14P	3:1	1.94 (0.09) bA	194.29 (19.23) aA	2.65 (0.06) bA	204.19 (6.80) bB	4.22 (0.16) dA		3.64 (0.15) aB	319.21 (21.19) aB	2.50 (0.07) aA	144.79 (12.22) aA	4.23 (0.09) cA
42N/42P	1:1	1.98 (0.09) bcA	195.37 (1.14) aA	3.15 (0.06) cA	274.70 (28.42) cA	3.57 (0.07) cB		8.28 (0.18) bB	718.41 (56.15) bB	5.20 (0.44) bB	310.98 (40.35) bA	1.94 (0.09) bA
42N/126P	1:3	2.40 (0.11) cA	230.98 (16.88) aA	3.48 (0.12) cA	301.69 (33.66) cA	3.07 (0.12) bB		13.91 (0.88) cB	1372.26 (57.74) cB	8.68 (0.14) cB	543.70 (58.93) cA	1.07 (0.07) aA
42N/252P	1:6	3.01 (0.26) dA	315.91 (44.54) bA	4.35 (0.17) dA	330.30 (34.81) cA	2.63 (0.25) aB		19.77 (0.76) dB	2194.79 (118.84) dB	11.63 (0.68) dB	568.61 (43.02) cB	0.81 (0.02) aA

[z]Includes leaves, stems, and inflorescences (if present).
[y]Includes rhizomes.

(Konnerup et al., 2009). P concentration reported by Mills and Jones (1996) was similar to our N42:P14 treatment. In a four-month microcosm study in Florida, DeBusk et al. (1995) reported that water canna (*Canna*. *flaccida* Salisb.) receiving "enriched" (75.7 $mg \cdot L^{-1}$ N, 29.2 $mg \cdot L^{-1}$ P) and "unenriched" (9.7 $mg \cdot L^{-1}$ N, 1.7 $mg \cdot L^{-1}$ P) were similar to the above-ground tissues N42:P7and N42:P14 for 20, 40, 60 d dairy wastewater for 20, 40, and 60 d accumulated N and P concentrations similar to that of *Canna* 'Bengal Tiger' in our study.

The high P shoot and root concentrations of Canna in the 42N:252P treatment--19.8 and 11.6 mg·g-1, respectively, were significantly higher than the 3.0 and 4.4 mg·g-1 in cattail shoot and root P, respectively. These high shoot and root P concentrations for Canna 'Bengal Tiger' have not been previously reported. Delorme et al. (2000) suggested that an attractive "P hyperaccumulator" must exhibit high P content (tens of g of P per kg dry weight or higher), high biomass, and have some form of post-harvest economic value. Delorme et al. (2000) found highest shoot P content in collard and corn (6.3 mg·g-1 and 4.9 $mg \cdot g^{-1}$, respectively) in response to growing on a high P soil (572.6 $mg \cdot kg^{-1}$ P), less than their anticipated foliar P concentration of 20 $mg \cdot g^{-1}$. The exceptionally high shoot P content in Canna and the ability to actively translocate a particular element from the root to the shoot—one of the definitions of a hyperaccumuator, makes it a suitable candidate for further research in remediating high P wastewater. N concentrations were within the range reported by Boyd and Hess (1970) for natural stands of *Typha latifolia* sampled from 30 sites farm ponds, vernal ponds, drainage ditches, and swamps in Georgia, Alabama, Mississippi, and Florida for all treatments. Aboveground N and P

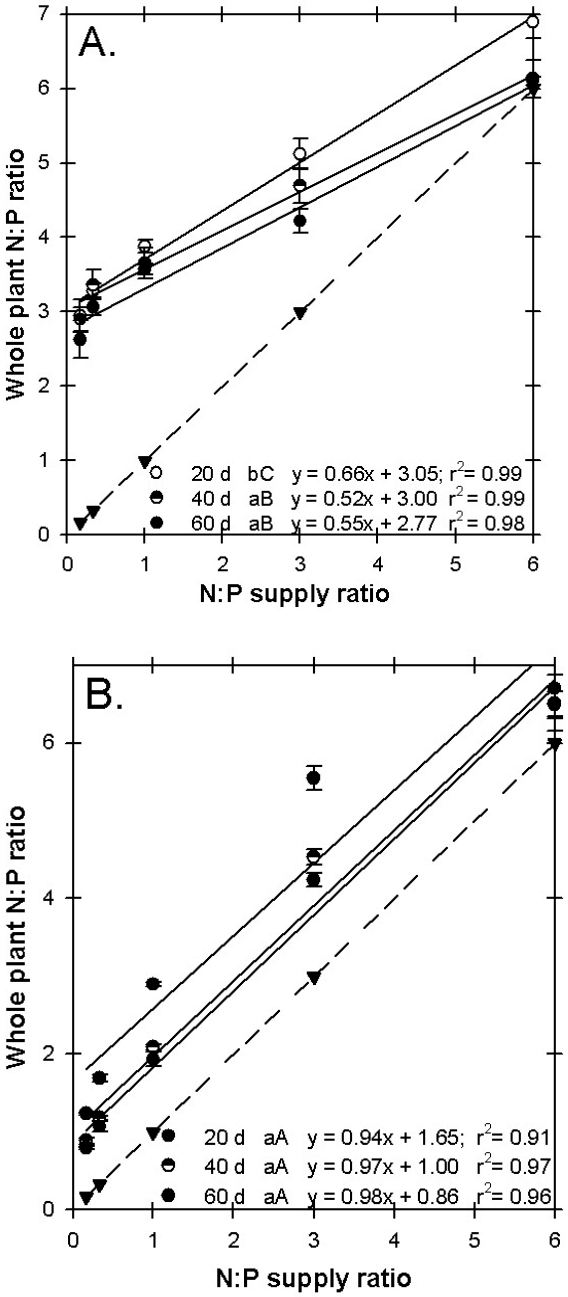
values for *Typha latifolia* were within the range reported by Ulrich and Burton (1988) in a microcosm study using varying combinations of NO_3-N and PO_4-P that ranged from 0 to 93.6 $g \cdot m^{-2}$ and 0 to 25.0 $g \cdot m^{-2}$, respectively.

Whole plant N:P concentration of *Canna* and *Typha* declined with increasing levels of P (Table 5.4). Whole plant N:P ratios of *Typha* were higher than *Canna* at every harvest date in the N42:P42, 42N:126P, and 42N:252P treatments, which occurred as a result of the higher P accumulation of *Canna* in contrast to *Typha*. Also, whole plant N:P concentration of both species were linearly correlated with N:P ratios of nutrients supplied in solution (Figure 5.6), similar to the findings of Zhang et al. (2008a) who found a linear correlation of individual plant tissues of *Canna indica* Linn. with N:P ratios of nutrients supplied in growth medium. *Canna* 'Bengal Tiger' whole plant N:P concentration differed from *Typha* at each harvest, and more closely matched the N:P ratios of the treatments than cattail.

Canna and *Typha* N tended to be partitioned in shoots at all treatment levels across all harvest dates. The pattern in *Canna* was regardless of N:P ratio, > 64% of N was in shoots at 20, 40, and 60 d 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). Similarly in *Typha* N:P ratio had no effect on N accumulation in shoots with > 64% allocated to shoots in all treatments.

Shoot P content increased with increasing concentrations of P in both species. In every treatment *Canna* shoot P content was > 65% at each harvest, similar to the findings with *Canna indica* Linn. with more than 56% of N and P located in aboveground tissues

Figure 5.6. Effect of N:P treatments at 20, 40, and 60 d on whole plant N:P concentration of *Typha latifolia* and *Canna* 'Bengal Tiger' (A and B, respectively) vs. N:P ratios of 5 treatments ($mg \cdot L^{-1}$): 6:1 (N42:P7); 3:1 (N42:P14); 1:1 (N42:P42); 1:3 (42N:126P); and 1:6 (42N:252P). Vertical bars = ± SE. Data points are the means of 4 plants. The dashed line represents N:P supply ratio = whole plant N:P ratio. Slopes of the regression lines were compared using linear contrasts and F tests. Lowercase and uppercase letters indicate significantly different slopes ($P \leq 0.05$) within and between species, respectively.



in response to high P levels (Zhang et al., 2008a). Unlike *Canna*, *Typha* shoot P declined across all treatments with > 52% in shoots at 20 d, > 47% in shoots at 40 d, and > 42% in shoots at 60 d. This increase in *Typha* root P accumulation can be explained on the basis of competition or sink/source relationship between root and shoot. The low N:P ratios were N-limiting in *Typha*, and since the root is the plant source for N, a low N concentration in nutrient solution restricts shoot growth more than root growth (Brouwer and de Wit, 1968). The reduced sink strength of *Typha* shoots resulted in the increase in root P assimilation, despite no statistical difference in biomass allocation between treatments, which supports the findings of other wetland studies where N and P accumulation did not reflect patterns of biomass allocation (Zhang et al., 2008a). There was no difference in total, shoot, or root dry weight among the five treatments, the greater P content in *Canna* and *Typha* at the 42N:127P and 42:254 treatments shows luxury consumption and storage, which has been demonstrated by other aquatic species (Spangler et al., 1976; Kadlec and Tilton, 1979; Cronk and Fennessy, 2001).

Table 5.5 shows average dry weight and N and P accumulation at 20, 40, and 60 d. Plant nutrient uptake (N and P) is the accumulation of each nutrient during the study, i.e., the difference in nutrient content from initial harvest to final harvest. Whole plant dry weight increased in both species at each harvest date, but generally no significant difference between treatments in either species (Table 5.5). No difference in whole plant dry weight between species at 20 and 40 d, but at 60 d *Typha* had significantly greater whole plant dry weight than *Canna* in the N42:P7 and N42:P14 treatments. *Typha's* higher biomass production at the high N:P ratios contrasted with *Canna's* ability to

Table 5.5. Effect of N:P treatments on biomass and nutrient uptake by *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days of treatment (July to September 2008) (n=4). Means (± SE) within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date.

Typha latifolia		20 days			40 days			60 days		
Treatment										
N/P ($mg \cdot L^{-1}$)	N:P	Whole plant dry wt. uptake (g)	Whole plant N uptake (mg)	Whole Plant P uptake (mg)	Whole plant dry wt. uptake (g)	Whole plant N uptake (mg)	Whole Plant P uptake (mg)	Whole plant dry wt. uptake (g)	Whole plant N uptake (mg)	Whole Plant P uptake (mg)
42N/7P	6:1	28.33 (6.46) aA	221.34 (60.04) abA	31.77 (4.90) aA	91.24 (9.78) abA	662.67 (66.89) aA	115.35 (4.88) aA	152.68 (15.16) aB	1121.62 (128.14) aB	177.53 (9.76) aA
42N/14P	3:1	31.83 (9.99) aA	208.98 (87.65) abA	78.90 (8.58) abA	87.18 (12.32) abA	703.72 (96.28) aA	216.71 (27.13) bA	135.37 (9.10) aB	988.43 (48.43) aA	299.37 (20.92) bA
42N/42P	1:1	31.57 (10.33) aA	226.77 (70.80) abA	136.33 (21.35) bcA	88.88 (16.04) abA	640.64 (84.61) aA	266.83 (33.52) bA	145.21 (11.82) aA	988.01 (70.60) aA	370.96 (27.39) bcA
42N/126P	1:3	37.59 (15.16)aA	256.15 (61.71) bA	176.59 (30.37) cdA	76.96 (12.05) aA	582.82 (81.68) aA	283.66 (39.83) bA	141.96 (14.14) aA	935.12 (85.62) aA	433.56 (47.60) cA
42N/252P	1:6	30.92 (7.73) aA	183.16 (65.08) aA	204.00 (45.34) dA	97.78 (14.38) bA	666.25 (80.70) aA	423.27 (66.05) cA	139.04 (13.59) aA	964.66 (57.67) aA	547.10 (70.55) dA

Canna 'Bengal Tiger'		20 days			40 days			60 days		
Treatment										
N/P ($mg \cdot L^{-1}$)	N:P	Whole plant dry wt. uptake (g)	Whole plant N uptake (mg)	Whole Plant P uptake (mg)	Whole plant dry wt. uptake (g)	Whole plant N uptake (mg)	Whole Plant P uptake (mg)	Whole plant dry wt. uptake (g)	Whole plant N uptake (mg)	Whole Plant P uptake (mg)
42N/7P	6:1	26.21 (1.30) aA	219.72 (57.61) aA	40.29 (6.35) aA	69.93 (2.53) aA	765.87 (65.09) aA	129.86 (5.87) aB	107.33 (2.94) abA	1113.79 (49.02) aA	184.05 (10.97) aA
42N/14P	3:1	36.10 (2.70) bA	351.84 (30.13) bcA	98.48 (7.82) aA	72.06 (2.20) aA	750.12 (59.61) aA	231.48 (8.07) aA	102.67 (3.30) aA	1148.44 (47.11) aA	350.04 (11.38) aA
42N/42P	1:1	37.34 (1.79) bA	318.16 (18.82) bcA	277.03 (4.20) bB	73.31 (3.27) aA	733.44 (28.51) aA	627.82 (14.60) bB	102.81 (7.50) aA	1171.75 (83.88) aA	915.44 (67.58) bB
42N/126P	1:3	34.20 (3.67) bA	274.21 (45.08) abA	532.01 (28.40) cB	69.72 (1.83) aA	739.76 (37.62) aA	1208.28 (31.62) (cB	118.64 (5.49) bA	1222.32 (83.72) aA	1802.02 (112.39) cB
42N/252P	1:6	41.06 (1.50) bA	369.98 (22.11) cB	847.46 (43.48) dB	68.82 (8.71) aA	798.50 (55.32) aA	1712.31 (133.33)dB	117.05 (4.64) abA	1320.68 (50.56) aB	2649.45 (123.54) dB

[z]Includes leaves, stems, and inflorescences (if present).

[y]Includes rhizomes.

assimilate higher levels of P at the lowest N:P treatments.

Whole plant N uptake showed no significant trends between treatments of each species or between species. Whole plant P uptake increased in both species at each treatment with increasing P levels and at each harvest date. *Canna* whole plant P uptake was greater than cattail at every harvest date in the three highest P treatments. At 60 d whole plant P uptake of *Canna* was 2.5-, 4-, and 5-fold higher than *Typha* in the N42:P42, 42N:126P, and 1N:6P treatments, respectively.

As P increased relative to a constant level of N, *Canna* acquired more P than *Typha*. Nitrogen source may have affected N and P uptake by *Typha*, since NH_4^+ is the predominant form of inorganic N in acidic, waterlogged, wetland soils (Mitsch and Gosselink, 2007) and is the preferred form of inorganic N for most wetland macrophytes (e.g., Brix et al., 2002; Tylova-Munzarova et al., 2005; Fang et al., 2007; Jampeetong and Brix, 2009). Nevetheless, *T. latifolia* produces optimal growth with either NH_4^+ or NO_3^- at pH 5.0-7.0 (Brix et al., 2002), but with NH_4^+ *T. latifolia* has a higher relative growth rate, greater tissue concentration of major nutrients, greater content of adenine nucleotides, and a higher affinity for inorganic N uptake than with NO_3^-. In a study of four wetland plants, Fang et al. (2007) observed that *Bacopa monnieri* and *Azolla* spp. prefer NO_3–N, whereas *Ludwigia repens* requires both N forms and *Canna indica* shows no preference for N forms (Zhang et al., 2009).

Mass Balance

Nitrogen (% content) for *Typha* was generally similar between treatments at each harvest date (Table 5.6). Nitrogen content was more variable between treatments for *Canna*, but levels were not different compared to *Typha*. Highest P recovery in *Canna* and *Typha* were the N42:P7 and N42:P14 treatments. *Typha* P content decreased 58% at 40 and 60 d in the N42:P42 treatments. At the N42:P42, 42N:126P, and 42N:252P treatments, *Canna* absorbed significantly more P than cattail at every harvest date with 2- to 5-fold differences between species. Less P remained in the *Canna* effluent at these 3 treatments than with *Typha* at 40 and 60 d. Generally, no treatment differences in unrecovered P occurred within and between species. *Canna* P removal exceeded *Typha* at the low N:P ratios. The improved efficiency was due to luxury consumption of P and storage predominantly in above-ground *Canna* tissues. Luxury consumption is defined as increased nutrient content and concentration with no change in plant weight (Aerts and Chapin, 2000).

Greater P removal in vegetated microcosms over unvegetated microcosms confers with many other studies that showed that vegetated wetland treatment beds remove a greater percentage of P from wastewater than gravel beds alone (Yang et al., 2001; Huett et al., 2005; Brisson and Chazarenc, 2009). Phosphorus uptake can be attributed to plant uptake (Brix, 1993) and bacterial uptake because plant rhizospheres support larger populations of bacteria than gravel media alone (Hatano et al. 1993). The presence of oxidized rhizospheres around plant roots also may have increased phosphorous removal by enhancing adsorption reactions that occur under oxic conditions

Table 5.6. Nitrogen and phosphorus mass balance (% of input) in *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40, and 60 d of treatment (n=4). Means within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest d. Uppercase letters indicate differences between species at each treatment at each harvest d.

20 days								
Typha latifolia								
Treatment		N content uptake (%)	Effluent N (%)	Unrecovered N (%)		P content uptake (%)	Effluent P (%)	Unrecovered P (%)
N/P ($mg \cdot L^{-1}$)	N:P							
42N/7P	6:1	51.9 aA	0.5 cA	47.6 aA		43.7 dA	4.2 aA	52.1 bA
42N/14P	3:1	48.4 aA	0.2 bA	51.4 aA		56.3 eA	2.2 aA	41.5 abA
42N/42P	1:1	53.4 aA	0.1 bA	46.5 aA		32.5 cA	32.9 bB	34.6 aA
42N/126P	1:3	70.5 aA	0.1 aA	29.4 aA		13.4 bA	52.1 cA	34.5 aA
42N/252P	1:6	44.4 aA	0.1 aA	55.5 aB		8.1 aA	61.5 cB	30.4 aA

Canna 'Bengal Tiger'								
Treatment		N content uptake (%)	Effluent N (%)	Unrecovered N (%)		P content uptake (%)	Effluent P (%)	Unrecovered P (%)
N/P ($mg \cdot L^{-1}$)	N:P							
42N/7P	6:1	50.7 aA	0.4 cA	48.9 cA		54.8 bA	2.4 aA	42.8 bA
42N/14P	3:1	80.4 bcA	0.1 abA	19.5 abA		70.0 cA	2.2 aA	27.8 aA
42N/42P	1:1	73.9 bcA	0.2 bA	25.9 bcA		65.4 bcB	11.1 bA	23.5 aA
42N/126P	1:3	64.9 abA	0.1 abA	35.0 bcA		40.7 aB	36.7 cA	22.6 aA
42N/252P	1:6	89.1 cA	0.1 aA	10.8 aA		33.8 aB	41.1 cA	25.1 aA

(Wolstenholme and Bayes, 1990).

Over the course of the experiment unrecoverable N ranged from 11 to 49% in *Canna* and 26 to 56% in *Typha* (Table 5.6). Unrecovered N was due to immobilization and denitrification by microorganisms or volatilization (Vymazal, 2007). Denitrification is a significant removal mechanism in both planted and unplanted systems (Breen, 1990). Nitrogen adsorption to gravel could have occurred to a certain degree, as found in other mass balance studies using gravel and sand substrates (Breen, 1990; Burgooon et al.,

Table 5.6. Nitrogen and phosphorus mass balance (% of input) in *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40, and 60 d of treatment (n=4). Means within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest d. Uppercase letters indicate differences between species at each treatment at each harvest d (continued).

40 days								
Typha latifolia								
Treatment		N content uptake (%)	Effluent N (%)	Unrecovered N (%)		P content uptake (%)	Effluent P (%)	Unrecovered P (%)
N/P ($mg \cdot L^{-1}$)	N:P							
42N/7P	6:1	64.6 aA	0.2 cA	35.1 aA		73.0 cA	1.9 aA	25.1 aA
42N/14P	3:1	73.6 aA	0.1 bA	26.3 aA		69.9 cA	5.3 bA	24.8 aA
42N/42P	1:1	66.8 aA	0.1 abA	33.2 aA		29.2 bA	41.7 cB	29.1 aB
42N/126P	1:3	58.0 aA	0.1 aA	42.0 aA		9.8 aA	61.4 dB	28.8 aA
42N/252P	1:6	69.4 aA	0.1 aA	30.6 aA		7.5 aA	59.1 dB	33.3 aB

Canna 'Bengal Tiger'								
Treatment		N content uptake (%)	Effluent N (%)	Unrecovered N (%)		P content uptake (%)	Effluent P (%)	Unrecovered P (%)
N/P ($mg \cdot L^{-1}$)	N:P							
42N/7P	6:1	76.7 aA	0.4 cB	22.9 aA		81.1 dA	1.6 aA	17.4 aA
42N/14P	3:1	78.4 aA	0.2 bA	21.5 aA		74.4 cdA	1.0 aA	24.6 aA
42N/42P	1:1	76.0 aA	0.1 abA	23.9 aA		68.3 cB	11.4 bA	20.4 aA
42N/126P	1:3	73.4 aA	0.1 aA	26.6 aA		41.8 bB	37.1 cA	21.1 aA
42N/252P	1:6	82.8 aA	0.1 aA	17.2 aA		30.4 aB	45.1 dA	24.6 aA

1991) coupled with the relatively high plant growth rate and a relatively low N loading rate compared to P loading rate. A mass balance study by Zhang et al. (2007) recovered 6 and 23% of sorbed N in the unvegetated, washed river sand "high" and "low" nutrient treatments, respectively, and 15 and 23% in the *Canna indica* high and low treatments and 19 and 33% sorbed P in the unplanted microcosms. Depletion of N could have also resulted from assimilation by the thin film of algae present near the gravel surface and from biofilm--single cells or pools of microorganisms embedded in a matrix of

Table 5.6. Nitrogen and phosphorus mass balance (% of input) in *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40, and 60 d of treatment (n=4). Means within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest d. Uppercase letters indicate differences between species at each treatment at each harvest d (continued).

60 days								
Typha latifolia								
Treatment		N content uptake (%)	Effluent N (%)	Unrecovered N (%)		P content uptake (%)	Effluent P (%)	Unrecovered P (%)
N/P ($mg \cdot L^{-1}$)	N:P							
42N/7P	6:1	73.9 aA	0.3 cA	25.8 aA		72.2 eA	2.4 aA	25.3 aA
42N/14P	3:1	68.1 aA	0.2 bA	31.7 aA		61.9 dA	9.8 bB	28.2 aA
42N/42P	1:1	67.6 aA	0.1 bA	32.3 aA		26.0 cA	46.9 cB	27.0 aA
42N/126P	1:3	62.5 aA	0.1 aA	37.4 aA		9.7 bA	63.8 dB	26.4 aA
42N/252P	1:6	65.4 aA	0.1 aA	34.6 aB		6.2 aA	67.8 dB	25.9 aB

Canna 'Bengal Tiger'								
Treatment		N content uptake (%)	Effluent N (%)	Unrecovered N (%)		P content uptake (%)	Effluent P (%)	Unrecovered P (%)
N/P ($mg \cdot L^{-1}$)	N:P							
42N/7P	6:1	73.7 aA	0.4 dA	25.9 bA		75.3 dA	4.0 aA	20.7 aA
42N/14P	3:1	78.8 abA	0.2 cA	21.1 abA		72.1 cdA	1.1 aA	26.8 aA
42N/42P	1:1	79.7 abcA	0.1 cA	20.2 abA		63.8 cB	12.2 bA	24.1 aA
42N/126P	1:3	82.2 bcA	0.1 bA	17.7 abA		40.7 bB	38.7 cA	20.6 aA
42N/252P	1:6	87.5 cB	0.1 aA	12.4 aA		30.6 aB	49.3 cA	20.1 aA

microbial-derived polymers attached to the gravel substrate (Zhang and Bishop, 1994; Consteron et al., 1995; Bigambo and Mayo, 2005). There is also evidence in the literature that N drawdown in soilless media leads to a high percentage of N immobilization and denitrification by microorganisms, which is manifested as unrecoverable or unaccountable N in mass balance studies.

Unrecovered P ranged from 17 to 43% *Canna* and from 25 to 42% in *Typha*, possibly due to formation of biofilm that was not quantified in the present study.

Breen (1990) found that biofilm represented a total of 11 and 10% of the influent N and P, respectively. Although comparatively small, the complex films of microbial cells and organic materials form the interface between plant and substratum surfaces and the interstitial water and influent load. As such, this component is directly involved in the transformation and availability of nutrients.

Direct comparison of N and P recovery by the aquatic garden plants in this study with other investigations is precluded by differing hydraulic characteristics, such as retention time, water level depth, and wastewater loading, along with differences in species compositions and densities, media, and design and size of the systems. Care must be exercised when attempting to to generalize the results from the microcosm or mesocosm experiments to field-scale wetlands, especially with regard to the quantitative role of plant uptake as a nutrient removal mechanism (Busnardo, et al., 1992). For illustrative purposes, the removal efficiencies of N and P (Table 5.6) were comparable to reported studies on other plant species. For example, the removals of total N and P in an artificial wetland were 95 and 99% of the input, respectively (Breen, 1990). In pilot-scale constructed wetlands, the removal was 90% of total added N and 55% of added P (Ayaz and Akça, 2001). Huett et al. (2005) reported that > 96% of added N and P was removed from simulated nursery runoff by the planted small-scale subsurface flow wetlands during the 19-month study period. In the microcosm, the high ratio of plant biomass to wetland volume is likely to have enhanced contact between plant roots and wastewater, thus providing a relatively large plant sink for nutrients. However, the high

N and P removal recorded by Headley et al. (2001, 2005) in a large-scale wetland during the establishment phase indicated that scale was not the sole factor.

Phosphorous removal is typically highly variable in constructed wetlands systems, ranging from 0–98% (Steiner and Freeman 1989; Watson et al. 1989). In a review of various vegetated constructed wetlands, Vymazal (2007) reported total N removal between 40 and 55% with removed load ranging between 250 and 630 g $N \cdot m^{-2} \cdot yr^{-1}$. Total P removal varied between 40 and 60% with removed load ranging between 45 and 75 g $P \cdot m^{-2} \cdot yr^{-1}$.

The high capacity for P-absorption by *Canna* was due to physical and physiological mechanisms. *Canna indica* possesses a fibrous root system; its rhizomes lack aerenchyma and are primarily used for storage (Chen et al., 2007). In a study of eight wetland plant species, *C. indica* had the most developed root system compared to other species studied, with the highest number of roots and root surface area compared to the rhizomatous helophytes *Acorus calamus*, *Hymenocallis littoralis*, *Phragmites communis*, and *Typha angustifolia* (Chen et al., 2007). Chen et al. (2007) observed that root growth is significantly faster, and root surface area considerably larger, with fibrous than with rhizomatic root systems in wastewater culture systems.

In a comparative kinetic uptake study of NH_4-N, NO_3-N, and P uptake, Zhang et al. (2009) found that the P uptake rate constant (K_m) of *Canna indica* was higher (157 $\mu mol \cdot L^{-1}$) than *S. validus* (60 $\mu mol \cdot L^{-1}$), showing that *Canna's* capacity for P uptake is greater when P concentration in the substrate is relatively high.

Plant Tissue Concentrations of Other Nutrients

Both species received the same concentration of nutrients throughout the duration of the study. Species differed in their abilities to acquire and store these minerals (Appendix, Table D.2). K concentration was highest in *Canna* shoots at 40 and 60 d compared to *Typha*. Zinc concentration was highest at 60 d in both species at the three highest P treatments. Copper concentration was highest in *Canna* shoots and roots at every harvest date compared to *Typha*, and manganese was highest in *Canna* roots at every treatment level.

Sodium concentration was highest in *Canna* 'Bengal Tiger' shoots and roots at every treatment level (Appendix, Table D.2). The concentrations were less than the Na concentrations observed in an earlier study (Polomski et al., 2007). Sodium concentration in *Typha* was much lower than in *Canna*. *Typha latifolia* is a freshwater species that is widely distributed in North America; it has been found in wetlands or drainage channels that undergo frequent influx of brackish and/or salt water (McNaughton and Fullem, 1970; Grace and Harrison, 1986; Holm et al., 1997).

In summary, the results of this study shows that phosphorus uptake of *Canna* and *Typha* was affected by N availability. Plant uptake and incorporation into plant tissue was the major factor responsible for N and P removal. Determining the optimal N:P ratio of aquatic species in order to maximize P-absorption prior to exposure of wastewater should be a design parameter in the establishment of constructed wetlands or vegetative filters (Adler et al., 2008). In cases where N:P ratio of wastewater is suboptimal or N-limited, *Canna* 'Bengal Tiger' may be a more viable economic and environmentally

appropriate alternative to the invasive *Phragmites australis* (Marks et al., 1994; Saltonstall, 2002). Further work needs to be done in pilot scale constructed wetlands to determine the effects of N:P ratio on nutrient recovery, propagation and production, marketable plant quality, and harvestability of *Canna* 'Bengal Tiger.'

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CHAPTER VI

SUMMARY AND CONCLUSIONS

This research investigated the potential use of commercially available aquatic garden plants to provide a sustainable, cost-effective, and low maintenance remediation solution compared to conventional wastewater treatment technologies. The marketable value of these plants offsets their production costs in the remediation of N and P from wastewater. Fifteen commercially available aquatic garden plants were evaluated for their ability to recover N and P, and in a subsequent study the effect of N:P ratio in the influent on P-assimilation was investigated.

Plants with highly efficient N and P recovery rates, such as *Pontederia cordata* L. 'Singapore Pink.', Louisiana Iris hybrid 'Full Eclipse, ' *Thalia geniculata* f. *rheumoides* Shuey, and *Oenenathe javanica* (Blume) DC. 'Flamingo.' can be placed at the discharge end of constructed wetlands. *Canna* 'Bengal Tiger,' 'Yellow King Humbert,' *Thalia*, *Oenanthe*, and *Phyla* are best sited near the inflow end of constructed wetlands because they assimilate high N and P concentrations. Additionally, these species and cultivars may also be suited for subsurface-flow constructed wetlands in greenhouse production systems because of their ability to assimilate high volumes of nutrient-rich water, which reduces the amount of effluent that must be discarded; however, this will reduce the availability of recycled wetland-treated water for irrigation, which is an important water conservation practice.

Of the floating macrophytes evaluated in our study, water hyacinth (*Eichhornia crassipes* [Mart.] Solms.) exhibited the highest nitrogen uptake efficiency. Phosphorus

recovery rates were similar for the water hyacinth, water lettuce (*Pistia stratiotes* L.), and dwarf redstemmed parrotfeather (*Myriophyllum aquaticum* [Vell.] Verdc.). These floating aquatic macrophytes can be integrated with emergent macrophytes in a self-contained polycultural subsurface flow constructed wetland system that can be used to remediate runoff from nursery and greenhouse operations. Also, they may have an important role in greenhouse production in temperate areas where they can be cultivated indoors to assimilate NO_3^-, and soluble PO_4^{3-}, and heavy metal trace elements, which are often applied year-round. Similar to the cannas and *Thalia*, these floating plants have the ability to process high volumes of nutrient-rich water that reduces the amount of effluent that has to be discarded.

The N:P ratio study showed that phosphorus uptake of *Canna* and *Typha* was affected by N availability. Plant uptake and incorporation into plant tissue was the major factor responsible for N and P removal. Determining the optimal N:P ratio of aquatic species in order to maximize P-absorption prior to exposure of wastewater should be a design parameter in the establishment of constructed wetlands or vegetative filters. In situations where N:P ratio of wastewater is suboptimal or N-limited, *Canna* 'Bengal Tiger' may be a more viable economic and environmentally appropriate alternative to *Typha latifolia*.

This study supports the use of aquatic garden plants as aesthetic and economically viable alternatives to traditional, obligate wetland plants in constructed wetlands and the need for further investigation to optimize species selection, cycling time, and production system design. Hydraulic loading rates and retention times, and species-specific

tolerance of pesticides are other important areas that need to be examined to allow nursery and greenhouse producers with limited growing space to customize their remediation/production areas. Also, further work needs to be done in pilot scale constructed wetlands to determine the effects of N:P ratio on nutrient recovery, propagation and production, marketable plant quality, and harvestability of *Canna* 'Bengal Tiger' and other important aquatic garden species.

APPENDICES

Appendix A

Tissue Mineral Concentrations

Table A.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of of seven aquatic garden plants grown for eight 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. Treatments were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface.

Canna 'Bengal Tiger' Shoots								
Treatment[z]	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------mg·g^{-1}----------			----------mg·kg^{-1}----------				
1	16.97	7.47	1.40	41.83	9.92	566.92	79.92	5079.25
2	16.96	6.08	1.33	31.58	8.33	582.75	75.08	4968.58
3	15.69	7.15	1.57	32.92	7.25	487.75	167.25	9844.67
4	17.58	7.06	2.08	30.67	6.83	373.92	78.50	15504.17
5	22.97	6.83	3.23	27.17	6.83	276.67	102.75	20355.92
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------mg·g^{-1}----------			----------mg·kg^{-1}----------				
1	11.06	2.03	1.26	108.17	16.75	178.42	379.00	5591.83
2	10.90	1.93	1.32	37.00	15.17	197.92	464.25	6534.58
3	10.02	2.49	1.63	28.83	12.17	137.42	424.50	11849.17
4	11.28	3.22	2.13	21.33	9.42	109.00	362.83	18054.17
5	12.83	4.71	3.00	17.33	8.33	90.50	473.92	27980.33

[z]1 = 0.39 N mg·L^{-1}/0.07 P mg·L^{-1}; 2 = 1.75 N mg·L^{-1}/0.18 P mg·L^{-1}; 3 = 10.44 N mg·L^{-1}/1.86 P mg·L^{-1}; 4 = 21.57 N mg·L^{-1}/3.63 P mg·L^{-1}; 5 = 36.81 N mg·L^{-1}/6.77 P mg·L^{-1}; 1 mg·L^{-1} = 1 ppm.

Table A.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of of seven aquatic garden plants grown for eight weeks (continued).

Canna 'Yellow King Humbert' Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	19.30	11.68	2.78	40.83	5.33	1062.67	128.17	3613.50
2	19.78	11.76	2.89	42.25	5.42	1098.00	139.92	3829.42
3	19.31	9.15	2.50	41.58	8.50	831.83	118.75	10001.83
4	17.39	7.60	2.73	33.58	4.67	567.67	99.42	15728.83
5	20.48	7.28	3.06	25.83	4.33	364.58	103.50	16070.92
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	11.46	2.73	2.62	45.67	9.08	239.17	779.00	8032.08
2	11.81	2.92	2.69	44.58	8.92	175.25	1605.92	10118.92
3	10.24	2.88	2.49	40.25	7.17	145.50	638.42	14603.42
4	10.42	3.10	2.56	35.67	5.83	103.92	524.17	19330.58
5	12.47	4.35	3.26	18.00	4.42	68.42	756.00	24982.00

Colocasia esculenta 'Illustris' Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	11.06	4.88	1.74	55.17	5.25	454.25	178.42	1969.83
2	10.65	5.21	1.68	103.00	5.50	343.08	163.75	3720.58
3	12.02	6.28	1.89	36.50	4.08	280.17	202.75	5422.00
4	13.57	7.04	2.13	27.33	3.08	109.00	157.83	7404.92
5	16.53	8.74	2.64	21.42	3.00	23.58	124.83	10213.33
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	7.75	2.98	1.07	51.17	4.25	158.83	275.75	2263.25
2	7.78	3.38	1.15	96.50	4.33	132.92	298.83	3899.00
3	7.27	3.49	1.23	49.58	3.58	77.00	439.50	6601.58
4	7.48	3.80	1.36	26.00	2.92	39.42	333.67	9059.75
5	8.06	4.57	1.82	26.83	2.83	23.83	209.67	13559.75

Table A.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of of seven aquatic garden plants grown for eight weeks (continued).

Eleocharis dulcis Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	19.08	2.93	1.63	40.25	3.75	363.33	63.58	1572.42
2	18.73	2.54	1.46	32.92	3.75	285.08	51.25	2437.75
3	20.65	2.90	2.03	28.92	3.08	230.50	74.33	5319.17
4	22.45	2.95	2.28	22.08	2.33	226.58	52.50	6153.58
5	24.67	2.92	2.23	18.42	3.08	119.33	51.17	7830.58
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	7.58	1.24	0.72	24.67	8.67	173.83	2357.92	1656.58
2	7.12	1.50	0.74	23.00	8.50	133.75	2565.58	2859.42
3	7.73	2.12	0.85	17.83	6.42	61.42	1816.83	4850.75
4	10.16	2.46	1.03	17.50	6.17	64.08	1510.92	4834.17
5	13.88	3.08	1.22	16.92	7.00	40.50	815.08	5100.75

Louisiana iris hybrid ‘Full Eclipse’ Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	11.27	6.34	1.08	22.25	3.42	155.17	54.42	2444.58
2	11.69	6.58	1.20	20.33	3.83	179.25	51.58	3164.33
3	13.24	6.93	1.40	15.00	5.08	139.83	63.00	4434.25
4	18.05	10.00	2.09	22.58	3.08	84.58	73.08	5983.25
5	22.40	9.25	2.11	29.92	4.92	66.00	81.83	5828.83
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	8.82	5.11	1.73	121.00	10.50	263.08	892.50	14894.75
2	11.27	4.76	1.56	102.17	11.92	245.50	812.08	17888.83
3	14.62	4.48	1.79	48.25	7.92	121.58	592.08	23787.75
4	19.33	4.22	2.06	28.17	6.42	74.58	488.33	26278.00
5	26.91	5.19	2.22	30.83	7.25	58.75	466.50	24053.42

Table A.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of of seven aquatic garden plants grown for eight weeks (continued).

Peltandra virginica Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	14.28	11.18	1.53	32.25	2.42	593.33	282.50	7441.00
2	14.22	12.98	1.92	30.92	3.33	822.08	113.08	8010.83
3	15.98	13.24	1.88	30.17	2.25	557.25	108.50	12814.58
4	18.12	12.66	2.12	24.83	2.17	461.58	96.00	16414.67
5	17.45	11.01	1.98	19.58	1.67	242.33	103.17	20390.17
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	16.23	4.35	1.61	64.33	3.58	178.42	331.67	4437.67
2	17.05	4.61	1.91	69.08	4.33	171.67	328.33	5982.83
3	17.26	5.48	2.10	75.83	4.00	115.42	396.33	9485.75
4	19.33	6.62	2.28	62.42	3.92	82.83	476.67	10689.25
5	20.79	5.43	2.00	42.75	3.17	51.83	296.67	12581.17

Pontederia cordata 'Singapore Pink' Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	10.58	14.79	2.66	46.33	12.00	306.83	54.75	4631.67
2	10.73	15.39	2.92	42.75	12.67	289.92	71.67	5171.50
3	14.30	14.99	3.41	28.50	8.08	274.92	64.50	5976.67
4	18.39	14.03	3.72	26.33	6.92	227.00	112.75	7063.17
5	25.38	12.90	4.26	17.58	5.17	144.42	68.33	11530.67
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	8.12	5.34	0.67	90.75	18.58	66.00	751.83	5896.67
2	8.53	5.61	0.63	73.58	15.58	51.58	659.00	8086.33
3	7.33	5.68	0.73	48.08	13.92	47.50	706.58	10250.33
4	7.32	5.91	0.97	50.92	14.67	46.83	862.25	11779.25
5	7.78	7.30	1.49	40.75	17.75	36.83	733.67	15429.33

Appendix B

Tissue Mineral Concentrations

Table B.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of five aquatic garden plants grown for eight 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. Treatments were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface.

Oenanthe Shoots								
Treatment[z]	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------mg·g^{-1}----------			----------mg·kg^{-1}----------				
1	14.35	11.21	2.06	31.58	4.50	183.75	142.67	3271.92
2	13.43	10.39	1.93	57.58	4.25	123.42	113.58	4190.08
3	13.91	7.39	1.83	26.00	2.67	46.67	60.58	7393.83
4	17.88	9.16	2.52	23.75	3.42	39.83	78.83	6774.42
5	27.67	9.05	3.10	24.42	3.25	64.50	81.75	11075.00
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------mg·g^{-1}----------			----------mg·kg^{-1}----------				
1	9.32	5.43	3.42	60.00	9.33	335.00	932.25	10728.25
2	7.48	5.98	3.24	244.25	11.08	229.92	930.83	12755.08
3	7.36	7.79	4.94	47.42	16.42	130.92	802.58	17071.75
4	8.00	7.27	4.95	28.58	8.83	108.25	919.50	18799.33
5	11.23	9.49	6.63	20.33	8.00	149.92	855.83	21059.42

[z]1 = 0.39 N mg·L^{-1}/0.07 P mg·L^{-1}; 2 = 1.75 N mg·L^{-1}/0.18 P mg·L^{-1}; 3 = 10.44 N mg·L^{-1}/1.86 P mg·L^{-1}; 4 = 21.57 N mg·L^{-1}/3.63 P mg·L^{-1}; 5 = 36.81 N mg·L^{-1}/6.77 P mg·L^{-1}; 1 mg·L^{-1} = 1 ppm.

Table B.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of of seven aquatic garden plants grown for eight weeks (continued).

Phyla Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	8.77	6.63	1.71	12.17	3.33	307.50	71.42	6359.25
2	9.96	7.12	2.08	13.42	3.42	219.08	66.92	7753.08
3	12.36	7.22	2.38	12.42	3.08	129.58	64.33	10955.75
4	14.93	7.79	2.62	11.92	3.00	110.00	72.58	12301.25
5	19.18	7.91	3.06	13.58	3.50	93.50	144.75	15942.75
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	4.87	1.28	0.44	32.50	7.67	134.50	519.17	2014.00
2	5.29	1.60	0.50	31.08	8.50	87.58	533.75	3200.83
3	7.83	1.75	0.66	26.25	6.00	66.17	607.25	3997.67
4	9.94	1.95	0.84	27.17	9.83	67.42	479.42	3718.75
5	9.64	2.19	1.08	47.00	7.42	62.00	434.25	3763.50

Rhyncospora Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	8.77	6.63	1.71	12.17	3.33	307.50	71.42	6359.25
2	9.96	7.12	2.08	13.42	3.42	219.08	66.92	7753.08
3	12.36	7.22	2.38	12.42	3.08	129.58	64.33	10955.75
4	14.93	7.79	2.62	11.92	3.00	110.00	72.58	12301.25
5	19.18	7.91	3.06	13.58	3.50	93.50	144.75	15942.75
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	4.87	1.28	0.44	32.50	7.67	134.50	519.17	2014.00
2	5.29	1.60	0.50	31.08	8.50	87.58	533.75	3200.83
3	7.83	1.75	0.66	26.25	6.00	66.17	607.25	3997.67
4	9.94	1.95	0.84	27.17	9.83	67.42	479.42	3718.75
5	9.64	2.19	1.08	47.00	7.42	62.00	434.25	3763.50

Table B.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of of seven aquatic garden plants grown for eight weeks (continued).

Thalia Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	7.85	6.63	1.71	12.17	3.33	307.50	71.42	6359.25
2	9.96	7.12	2.08	13.42	3.42	219.08	66.92	7753.08
3	12.36	7.22	2.38	12.42	3.08	129.58	64.33	10955.75
4	14.93	7.79	2.62	11.92	3.00	110.00	72.58	12301.25
5	19.18	7.91	3.06	13.58	3.50	93.50	144.75	15942.75
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	4.87	1.28	0.44	32.50	7.67	134.50	519.17	2014.00
2	5.29	1.60	0.50	31.08	8.50	87.58	533.75	3200.83
3	7.83	1.75	0.66	26.25	6.00	66.17	607.25	3997.67
4	9.94	1.95	0.84	27.17	9.83	67.42	479.42	3718.75
5	9.64	2.19	1.08	47.00	7.42	62.00	434.25	3763.50

Typha minima Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	17.13	14.83	2.34	54.42	3.92	500.50	101.67	2697.17
2	18.97	14.38	2.52	51.17	3.92	477.25	97.17	3737.17
3	20.90	13.01	2.61	40.50	3.25	260.58	81.83	5665.75
4	23.72	11.13	2.84	32.83	3.58	154.50	73.08	7043.50
5	28.68	9.89	2.47	24.42	3.67	100.00	81.33	9887.58
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	10.21	3.46	1.16	79.08	12.25	168.58	1464.83	4439.25
2	10.07	3.88	1.32	76.17	11.75	156.67	1135.67	6632.00
3	11.48	4.43	1.70	58.83	12.17	100.33	1110.58	11309.58
4	11.77	4.63	2.13	51.17	11.67	77.17	787.83	13908.83
5	17.88	4.32	2.12	42.00	11.17	64.08	634.33	14584.17

Appendix C

Tissue Mineral Concentrations

Table C.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of three floating hydrophytes grown for eight 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. Treatments were initially batch-loaded and then supplied every two days to maintain the water level at the gravel surface.

Eichhornia crassipes Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	10.93	22.88	1.43	14.92	5.58	111.58	85.17	5803.67
2	10.57	19.10	1.43	12.83	4.58	114.42	50.75	6001.42
3	12.79	19.21	2.05	20.33	4.33	147.25	73.42	14392.50
4	14.71	14.96	2.53	15.58	4.08	157.58	57.08	16480.58
5	20.77	13.88	3.38	13.92	3.92	63.58	57.58	16526.83
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	5.58	9.30	1.40	24.83	7.00	84.33	659.17	11545.75
2	4.29	7.77	1.07	29.75	7.00	74.58	552.75	10288.42
3	5.32	8.53	1.33	135.33	7.33	56.17	570.42	16799.00
4	7.33	7.79	1.10	35.83	7.00	56.00	917.17	17376.33
5	7.45	9.76	1.33	18.50	7.00	52.00	1120.25	23043.92

[z]1 = 0.39 N $mg \cdot L^{-1}$/0.07 P $mg \cdot L^{-1}$; 2 = 1.75 N $mg \cdot L^{-1}$/0.18 P $mg \cdot L^{-1}$; 3 = 10.44 N $mg \cdot L^{-1}$/1.86 P $mg \cdot L^{-1}$; 4 = 21.57 N $mg \cdot L^{-1}$/3.63 P $mg \cdot L^{-1}$; 5 = 36.81 N $mg \cdot L^{-1}$/6.77 P $mg \cdot L^{-1}$; 1 $mg \cdot L^{-1}$ = 1 ppm.

Table C.1. Tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of three floating hydrophytes grown for eight weeks (continued).

Myriophyllum aquaticum Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	14.35	11.21	2.06	31.58	4.50	183.75	142.67	3271.92
2	13.43	10.39	1.93	57.58	4.25	123.42	113.58	4190.08
3	13.91	7.39	1.83	26.00	2.67	46.67	60.58	7393.83
4	17.88	9.16	2.52	23.75	3.42	39.83	78.83	6774.42
5	27.67	9.05	3.10	24.42	3.25	64.50	81.75	11075.00
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	9.32	5.43	3.42	60.00	9.33	335.00	932.25	10728.25
2	7.48	5.98	3.24	244.25	11.08	229.92	930.83	12755.08
3	7.36	7.79	4.94	47.42	16.42	130.92	802.58	17071.75
4	8.00	7.27	4.95	28.58	8.83	108.25	919.50	18799.33
5	11.23	9.49	6.63	20.33	8.00	149.92	855.83	21059.42

Pistia stratiotes Shoots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	23.75	13.18	3.18	66.58	5.08	259.08	121.50	13638.00
2	22.16	12.18	3.13	54.92	4.17	290.17	134.50	15866.75
3	20.95	11.71	2.96	55.33	4.25	195.33	75.25	18978.67
4	20.26	12.94	3.05	45.75	3.25	122.00	65.08	20638.08
5	25.55	16.79	4.00	39.25	53.17	76.42	128.17	25203.58
Roots								
Treatment	K	Ca	Mg	Zn	Cu	Mn	Fe	Na
	----------$mg \cdot g^{-1}$----------			----------$mg \cdot kg^{-1}$----------				
1	19.95	8.58	1.01	172.82	12.55	262.27	1829.00	5671.00
2	16.29	8.61	0.85	120.36	11.64	269.91	1458.00	6820.64
3	18.57	8.34	0.99	105.09	10.18	150.36	1094.27	9661.55
4	22.44	8.99	1.32	82.64	10.45	98.91	938.91	12480.00
5	21.50	10.04	2.99	50.82	10.09	93.27	931.73	16125.18

Appendix D

Influence of N:P Ratio on Height, Biomass, and Mineral Concentrations

Figure D.1. Effect of N:P treatments on average height of *Canna* 'Bengal Tiger' (A) and *Typha latifolia* (B) from July-August 2008. Each point is the mean of n=12 (day 4-20), n=8 (day 24-40), and n=4 (day 44-60). Vertical bars are ± SE; ns = not significant ($P \leq 0.05$) by LSD.

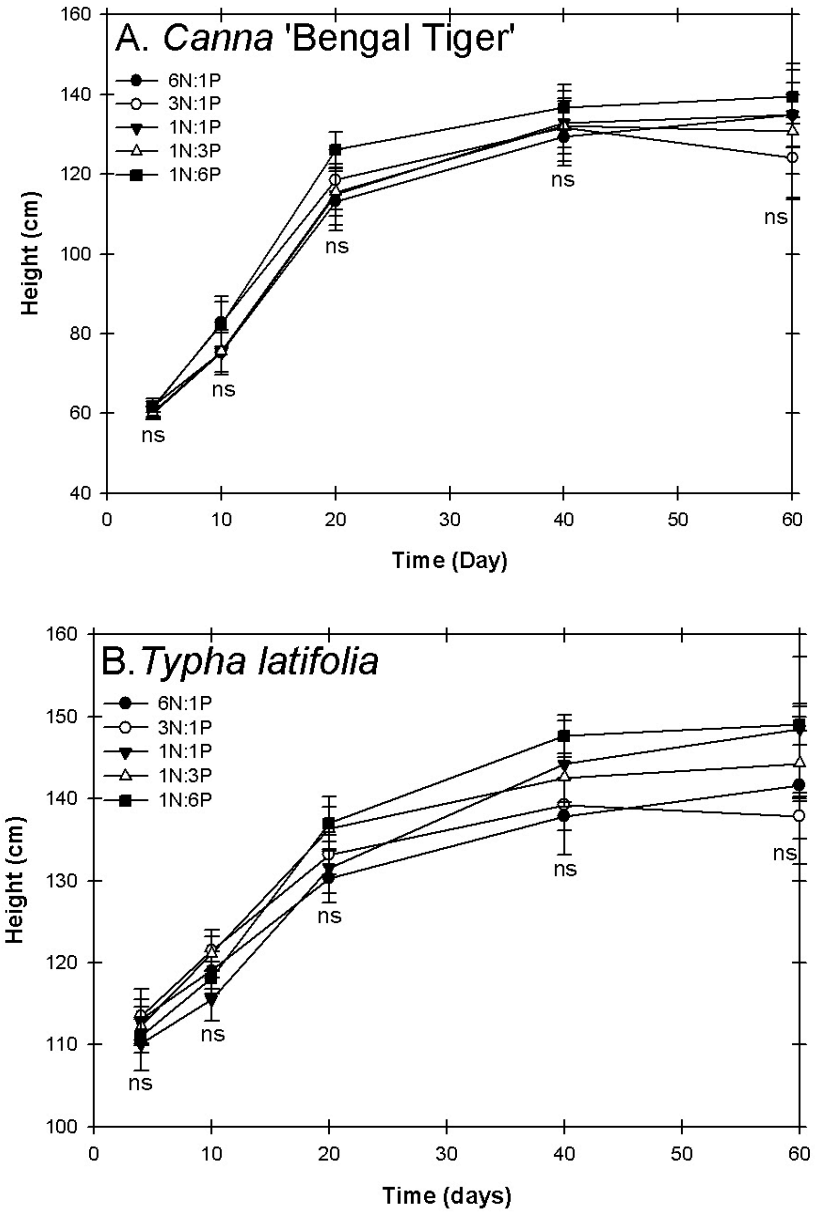

Figure D.2. Effect of N:P treatments on growth index (widest width measurement + perpendicular width measurement + height)/3) of *Canna* 'Bengal Tiger' from July-August 2008. Each point is the mean of n=12 (day 4-20), n=8 (day 24-40), and n=4 (day 44-60). Vertical bars are ± SE; ns = not significant ($P \leq 0.05$) by LSD.

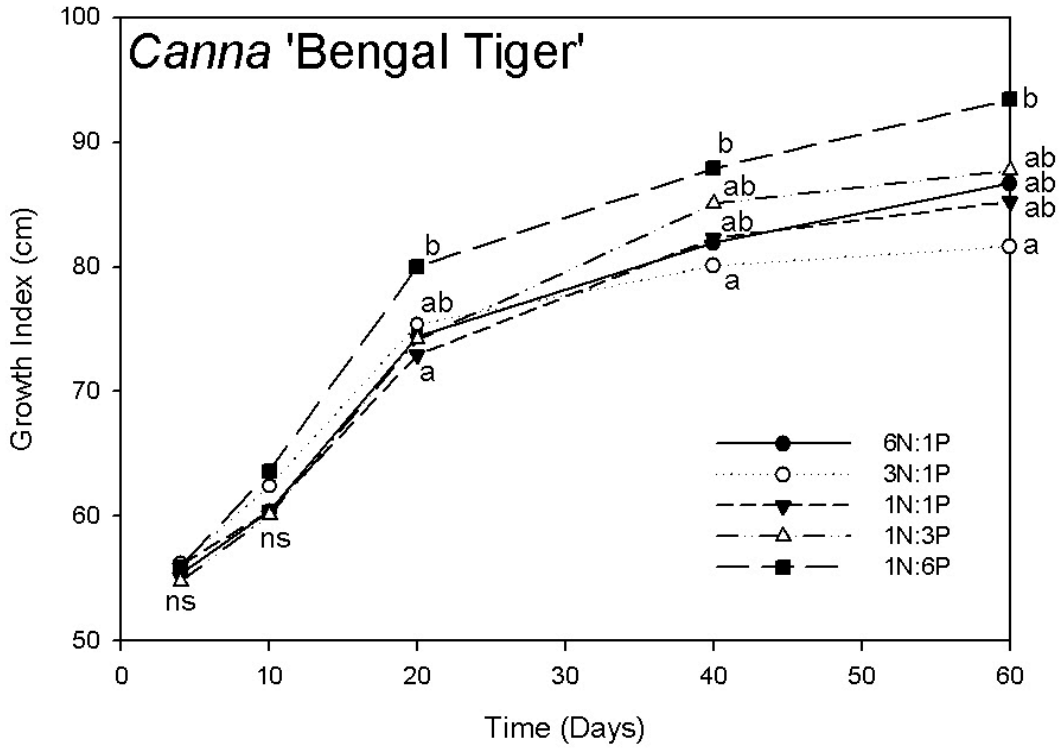

Figure D.3. Incremental P (mg) effluent (July-August) of *Typha latifolia* and *Canna* 'Bengal Tiger' (A and B, respectively). Vertical bars = ± SE. Each point is the mean of n=12 (day 4-20), n=8 (day 24-40), and n=4 (day 44-60).

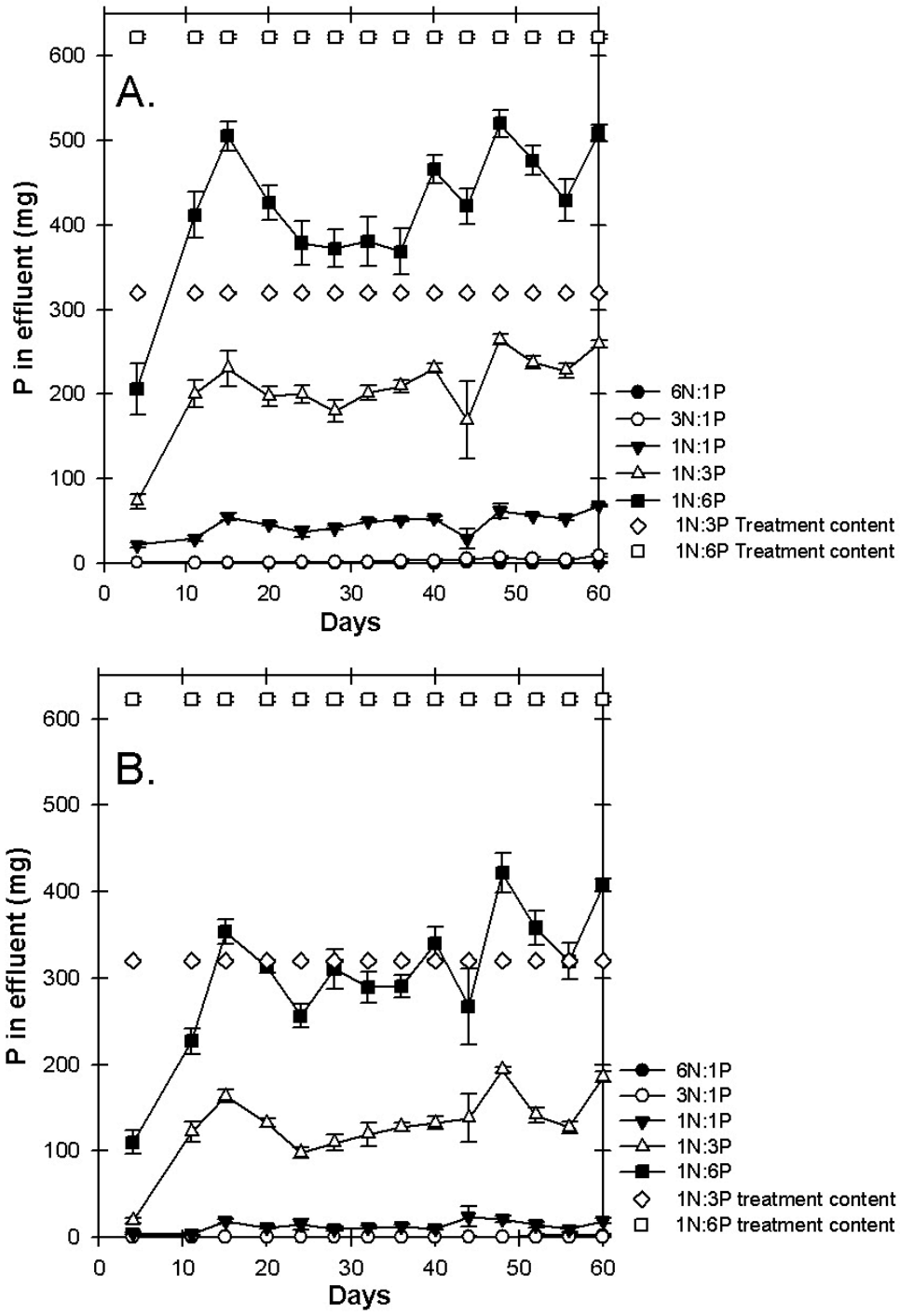

Table D.1. Effect of N:P treatments on biomass data areal basis in *Canna* 'Bengal Tiger' and *Typha latifolia* at 20, 40 and 60 days of treatment (n=4). Means within columns followed by the same letter are not significantly different ($P \leq 0.05$) by LSD. Lowercase letters indicate differences between treatments at each harvest date. Uppercase letters indicate differences between species at each treatment at each harvest date.

		Typha latifolia				*Canna* 'Bengal Tiger'		
20 d								
Treatment		Total dry wt. ($g \cdot m^{-2}$)	Shoot[z] dry wt ($g \cdot m^{-2}$)	Root[y] dry wt. ($g \cdot m^{-2}$)		Total dry wt. ($g \cdot m^{-2}$)	Shoot dry wt ($g \cdot m^{-2}$)	Root dry wt. ($g \cdot m^{-2}$)
N/P ($mg \cdot L^{-1}$)	**N:P**							
42N/P7	6:1	1464.90 aA	976.63 aA	488.26 aA		1456.11 aA	874.17 aA	581.94 aA
42N/14P	3:1	1538.52 aA	996.05 aA	542.47 aA		1664.30 bA	990.84 abA	673.46 abA
42N/42P	1:1	1533.15 aA	977.11 aA	556.05 aA		1690.35 bA	988.47 abA	701.87 bA
42N/126P	1:3	1659.77 aA	1003.68 aA	656.09 aA		1624.25 bA	953.16 aA	671.09 abA
42N/252P	1:6	1519.42 aA	952.95 aA	566.47 aA		1768.60 bA	1109.04 bA	659.56 abA
40 d								
		Total dry wt. ($g \cdot m^{-2}$)	Shoot dry wt ($g \cdot m^{-2}$)	Root dry wt. ($g \cdot m^{-2}$)		Total dry wt. ($g \cdot m^{-2}$)	Shoot dry wt ($g \cdot m^{-2}$)	Root dry wt. ($g \cdot m^{-2}$)
42N/P7	6:1	2789.18 abA	1630.78 aA	1158.40 abA		2376.28 aA	1419.11 aA	957.16 aA
42N/14P	3:1	2703.61 abA	1707.87 aA	995.74 aA		2421.17 aA	1522.05 aA	899.12 aA
42N/42P	1:1	2739.55 abA	1582.68 aA	1156.88 abA		2447.48 aA	1392.96 aA	1054.52 aA
42N/126P	1:3	2488.63 aA	1470.42 aA	1018.21 abA		2371.91 aA	1499.32 aA	872.59 aA
42N/252P	1:6	2926.74 bA	1687.45 aA	1239.29 bA		2352.91 aA	1490.16 aA	862.75 aA
60 d								
		Total dry wt. ($g \cdot m^{-2}$)	Shoot dry wt ($g \cdot m^{-2}$)	Root dry wt. ($g \cdot m^{-2}$)		Total dry wt. ($g \cdot m^{-2}$)	Shoot dry wt ($g \cdot m^{-2}$)	Root dry wt. ($g \cdot m^{-2}$)
42N/P7	6:1	4082.57 aB	2449.64 aA	1632.93 aB		3163.67 abA	1926.32 aA	1237.34 aA
42N/14P	3:1	3718.19 aB	2091.46 aA	1626.72 aB		3065.47 aA	1849.44 aA	1216.03 aA
42N/42P	1:1	3925.27 aA	2092.67 aA	1832.60 aA		3068.41 aA	1821.49 aA	1246.92 aA
42N/126P	1:3	3856.80 aA	2019.21 aA	1837.60 aA		3401.59 bA	2087.62 abA	1313.97 aA
42N/252P	1:6	3795.28 aA	2190.93 aA	1604.36 aB		3368.22 abA	2335.75 bA	1032.47 aA

[z]Includes leaves, stems, and inflorescences (if present).
[y]Includes rhizomes.

Table D.2. Effect of N:P treatments on the tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days (n=4) in a greenhouse experiment conducted from July-August 2008. Lowercase letters indicate differences between treatments at each harvest date ($P \leq 0.05$). Uppercase letters indicate differences between species at each treatment at each harvest date.

20 days									
Typha latifola									
Shoots									
Treatment		K	Ca	Mg	Zn	Cu	Mn	Fe	Na
N/P ($mg \cdot L^{-1}$)	N:P	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)
42N/P7	6:1	20.3 aA	6.1 aA	1.9 aA	77 aA	8 bA	276 aA	47 aA	5,221 aA
42N/14P	3:1	20.1 aA	6.7 aA	2.0 aA	71 aA	7 abA	272 aA	77 aA	6,448 bA
42N/42P	1:1	19.3 aA	6.4 aB	1.9 aA	65 aA	7 abA	304 aA	66 aA	5,260 aA
42N/126P	1:3	19.6 aA	6.7 aA	1.9 aA	71 aA	7 abA	282 aA	61 aA	5,579 abA
42N/252P	1:6	20.5 aA	6.8 aB	2.0 aA	72 aA	8 aA	319 aA	41 aA	6,125 abA
Roots									
Treatment		K	Ca	Mg	Zn	Cu	Mn	Fe	Na
N/P ($mg \cdot L^{-1}$)	N:P	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)
42N/P7	6:1	16.5 aA	4.0 abB	1.6abA	245 aA	58 aA	144 bA	371 aA	9,043 aA
42N/14P	3:1	15.8 aA	3.9 abB	1.5aA	251 aA	57 aA	127 abA	366 aA	9,831 aA
42N/42P	1:1	16.2 aA	4.4 bB	1.8 bA	220 aA	53 aA	135 abA	539 aA	8,422 aA
42N/126P	1:3	17.4 aA	4.3 abB	1.7 abA	226 aA	47 aA	114 aA	400 aA	9,339 aA
42N/252P	1:6	15.5 aA	3.7 aA	1.5 aA	192 aA	42 aA	114 aA	394 aA	8,843 aA

Table D.2. Effect of N:P treatments on the tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days (n=4) in a greenhouse experiment conducted from July-August 2008. Lowercase letters indicate differences between treatments at each harvest date ($P \leq 0.05$). Uppercase letters indicate differences between species at each treatment at each harvest date (continued).

40 days									
Typha latifolia									
Shoots									
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	K ($mg \cdot g^{-1}$)	Ca ($mg \cdot g^{-1}$)	Mg ($mg \cdot g^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Na ($mg \cdot kg^{-1}$)
42N/P7	6:1	17.5aA	6.9 abB	1.6 aA	87 bA	8 bA	382 abA	47 aA	4,654 aA
42N/14P	3:1	16.5 aA	6.5 aB	1.5 aA	79 bA	8abA	327 aA	99 aA	5,716 aA
42N/42P	1:1	17.1 aA	6.8 aB	1.5 aA	72 abA	8 abA	348 abA	51 aA	5,391 aA
42N/126P	1:3	18.2 aA	7.6 cA	1.6 aA	67 abA	7 abA	380 abA	82 aA	5,585 aA
42N/252P	1:6	16.4 aA	7.5bcA	1.5 aA	58 aA	7 aA	408 bA	58 aA	5,374 aA
Roots									
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	K ($mg \cdot g^{-1}$)	Ca ($mg \cdot g^{-1}$)	Mg ($mg \cdot g^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Na ($mg \cdot kg^{-1}$)
42N/P7	6:1	13.6 aB	3.9 aB	1.4 aA	205aA	60 aA	145 aA	326 aA	5,987 aA
42N/14P	3:1	13.1 aA	3.9 aA	1.5 aA	226 aA	64 aA	144 aA	380 aA	7,568 abA
42N/42P	1:1	13.3 aA	4.1 aA	1.5 aA	207 aA	60 aA	141 aA	373 aA	7,375 abA
42N/126P	1:3	15.0 aA	4.1 aA	1.5 aA	227 aA	76 aA	143 aA	451 aA	8,670 bA
42N/252P	1:6	13.8 aA	4.2 aA	1.6 aA	176 aA	51 aA	142 aA	319 aA	6,879 aA

Table D.2. Effect of N:P treatments on the tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days (n=4) in a greenhouse experiment conducted from July-August 2008. Lowercase letters indicate differences between treatments at each harvest date ($P \leq 0.05$). Uppercase letters indicate differences between species at each treatment at each harvest date (continued).

60 days									
Typha latifolia									
Shoots									
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	K ($mg \cdot g^{-1}$)	Ca ($mg \cdot g^{-1}$)	Mg ($mg \cdot g^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Na ($mg \cdot kg^{-1}$)
42N/P7	6:1	16.4aA	7.1 aA	1.4aA	95aA	10 cA	429 aA	62 aA	4,704 aA
42N/14P	3:1	16.3 aA	7.4 abB	1.4 aA	90 aA	9 bcA	418 aA	70 aA	5,242 aA
42N/42P	1:1	15.8 aA	7.9 abB	1.4 aA	84 aA	9 abA	449 abA	95 aA	5,804 aA
42N/126P	1:3	16.4 aA	8.6 bB	1.5 aA	72 aA	8aA	497 bA	51 aA	5,447 aA
42N/252P	1:6	17.8 aA	8.1 abA	1.4 aA	73 aA	8 abA	427 aA	36 aA	5,676 aA
Roots									
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	K ($mg \cdot g^{-1}$)	Ca ($mg \cdot g^{-1}$)	Mg ($mg \cdot g^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Na ($mg \cdot kg^{-1}$)
42N/P7	6:1	12.5 aA	3.3 aA	1.4 aA	205 aA	66 bA	154aA	438 aA	6,012 aA
42N/14P	3:1	12.7 aB	3.4 aB	1.4 aA	192 aA	56 abA	152 aA	334 aA	5,541 aA
42N/42P	1:1	12.5 aA	3.3 aA	1.3 aA	189 aA	56 abA	140 aA	355 aA	5,570 aA
42N/126P	1:3	13.2 aB	3.2 aA	1.3 aA	173 aA	50 aA	136 aA	357 aA	5,224 aA
42N/252P	1:6	12.9 aB	3.5 aA	1.4 aA	175 aA	54 abA	139 aA	405 aA	5,998 aA

Table D.2. Effect of N:P treatments on the tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days (n=4) in a greenhouse experiment conducted from July-August 2008. Lowercase letters indicate differences between treatments at each harvest date ($P \leq 0.05$). Uppercase letters indicate differences between species at each treatment at each harvest date (continued).

20 days									
Canna 'Bengal Tiger'									
Shoots									
Treatment		K	Ca	Mg	Zn	Cu	Mn	Fe	Na
N/P ($mg \cdot L^{-1}$)	N:P	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)
42N/7P	6:1	26.6 bA	5.2 abA	3.04 aB	83 bA	19 abB	343 aA	119 bA	11,978 abB
42N/14P	3:1	24.6 abA	5.4 abA	3.09 aB	65 aA	16 aB	325 aA	99 abA	10,784 aB
42N/42P	1:1	23.4 aA	5.1 aA	2.94 aB	72 abA	17 abB	347 aA	93 abA	12,040 abB
42N/126P	1:3	24.5 abA	5.9 bA	3.16 aB	72 abA	18 abB	375 aB	75 aA	13,630 cB
42N/252P	1:6	23.6 aA	5.7 abA	3.13 aB	62 aA	19 bB	388 aA	65 aB	13,297 bcB
Roots									
Treatment		K	Ca	Mg	Zn	Cu	Mn	Fe	Na
N/P ($mg \cdot L^{-1}$)	N:P	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)
42N/7P	6:1	13.3 bA	2.5 aA	2.7 aB	332 abA	117 aB	191 aA	375 aA	18,451 aB
42N/14P	3:1	12.1 abA	2.6 aA	2.4 aB	262 aA	92 aA	180 aB	376 aA	17,944 aB
42N/42P	1:1	12.3 abA	2.5 aA	2.6 aB	332 bB	102 aB	192 aB	605 bA	18,492 aB
42N/126P	1:3	11.3 aA	2.9 bA	2.6 aB	323 abA	109 aB	194 aB	440 abA	18,569 aB
42N/252P	1:6	11.4 aA	3.3 cA	2.7 aB	316 abB	108 aB	195 aB	489 abA	18,950 aB

Table D.2. Effect of N:P treatments on the tissue concentrations of K, Ca, Mg, Zn, Cu, Mn, Fe, and Na in the shoots and roots of *Typha latifolia* and *Canna* 'Bengal Tiger' at 20, 40 and 60 days (n=4) in a greenhouse experiment conducted from July-August 2008. Lowercase letters indicate differences between treatments at each harvest date ($P \leq 0.05$). Uppercase letters indicate differences between species at each treatment at each harvest date (continued).

40 days									
Canna 'Bengal Tiger'									
Shoots									
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	K ($mg \cdot g^{-1}$)	Ca ($mg \cdot g^{-1}$)	Mg ($mg \cdot g^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Na ($mg \cdot kg^{-1}$)
42N/P7	6:1	24.7 aB	4.9 abA	2.8 abB	80 aA	19 aB	384 aA	98 cB	13,281 aB
42N/14P	3:1	22.7 aB	4.5 aA	2.6 aB	68 aA	19 aB	376aA	91 bcA	13,944 abB
42N/42P	1:1	23.2 aB	5.2 bA	2.8 abB	80 aA	19 aB	418 abA	88 bA	15,627 bcB
42N/126P	1:3	23.3 aB	5.2 bB	2.9 abB	80 aA	22 aB	466 bcA	58 aA	17,139 cB
42N/252P	1:6	23.6 aB	5.9 cB	3.1 bB	82 aA	27 bB	523 cA	56 aA	19,142dB
Roots									
Treatment									
N/P ($mg \cdot L^{-1}$)	N:P	K ($mg \cdot g^{-1}$)	Ca ($mg \cdot g^{-1}$)	Mg ($mg \cdot g^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Na ($mg \cdot kg^{-1}$)
42N/P7	6:1	11.4 aA	2.9 aA	2.2 aB	292 abA	141 aB	196 aB	445 abA	22,146 abB
42N/14P	3:1	11.2 aA	2.9 aB	2.3 aB	274 aA	136 aB	204 aB	388 aA	22,272 abB
42N/42P	1:1	11.9 aA	3.0 aA	2.5 abB	285 abA	135 aB	214 abB	559 bB	21,583 aB
42N/126P	1:3	11.4 aA	3.4 bA	2.8 bcB	369 bcA	159 aA	250 bB	481 abA	23,945 bcB
42N/252P	1:6	11.8 aA	3.7 cA	2.9 cB	390 dA	167 aB	249 bB	438 abA	25,027 cB