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CONSTRUCTED WETLANDS FOR THE LANDSCAPE NURSERY INDUSTRY: NUTRIENT ASSIMILATION AND REMOVAL

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CONSTRUCTED WETLANDS FOR THE LANDSCAPE NURSERY INDUSTRY: NUTRIENT ASSIMILATION AND REMOVAL

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Environmental Toxicology

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by
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ABSTRACT

Runoff from nursery operations is considered a potential non-point source contamination. Water quality and quantity are quickly becoming important factors that drive management practices at these facilities. Constructed wetland systems (CWS) are a management tool that can be used by nursery operations to improve water quality both for recycling within nursery production areas and for eventual release from nursery production areas into surrounding surface waters. The overall goal of this research was to optimize nutrient removal efficiencies in CWS. To accomplish this goal, I characterized the following: (1) the P sorption and desorption capacity of several substrates; (2) the effect of hydraulic retention time (HRT) and nutrient loading rate on nutrient retention efficiency in surface-flow CWS; (3) P sorption by an industrial mineral aggregate in a secondary, subsurface-flow treatment; (4) the impact of CWS planting style, whether floating mat, rooted plant material, or horticulturally-significant species, on nutrient removal; and (5) brick and industrial mineral aggregate root-bed substrate P sorption capacity under stable nutrient loading rates.

The industrial mineral aggregate substrate displayed the greatest P sorption capacity of the substrates screened with a Langmuir S_{max} of 256.3 mg/kg P sorbed by the coarse aggregate (mesh size 4/20) and 462.9 mg/kg P sorbed by the fine aggregate (mesh size 24/48). Brick substrate (mesh size 4/20) exhibited substantially lower P sorption capacity with a S_{max} of only 6.79 mg/kg. The coarse aggregate sorbed 76% of the P in solution with exposure concentrations < 100 mg P/L, and so seemed ideally suited as a subsurface flow CWS root-bed substrate. The brick sorbed substantially less P but is also less expensive and, therefore, may be a viable root bed substrate.

Nutrient loading rate is very important. High nutrient inflow treatments were not adequately remediated with these experimental-scale systems, whereas the low and medium nutrient inflow treatments were efficiently assimilated. Hydraulic retention time was not a consistent factor influencing nutrient removal efficiency for N or P. The 4-day HRT resulted in consistently less P export from the CWS. Floating and rooted treatments demonstrated highly efficient N and P assimilation, while the horticulturally significant species were not as effective. Brick sorbed P efficiently but it saturated more quickly and did not reduce export concentrations as well as the industrial mineral aggregates, which effectively sorbed P from solution and reduced P exports from the mesocosms. The surface- to subsurface-flow CWS was effective at assimilating and fixing nutrients from simulated nursery runoff.

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PREFACE

Nutrient-rich runoff from nursery production areas is under increasing scrutiny because of the detrimental effects nutrient enrichment can cause in surface waters. An effective and reliable remediation strategy is needed to reduce nutrient escape from nursery operations. This research evaluated and defined some factors that contribute to nutrient remediation in constructed wetland systems. The overall goal was to optimize constructed wetland system design to maximize nitrogen and phosphorus remediation. To accomplish this goal, I formulated a number of objectives including: 1) characterize the phosphorus sorption capacity of several substrates, 2) evaluate the impact of hydraulic retention time on remediation efficiency, 3) assess the effect of nutrient loading rate effect on remediation efficiency, 4) characterize planting strategy to attain the highest nutrient removal efficiency, 5) compare P sorbing effectiveness of two substrates over time, and 6) evaluate the remediation efficiency of planted and non-planted subsurface-flow wetlands.

This dissertation consists of a literature review describing past and current work with constructed wetlands and nutrient remediation, and three journal articles. The first journal article (Chapter 2) describes phosphorus chemistry and the potential of several media for phosphorus sorption and desorption (objective 1). The second article (Chapter 3) evaluates the effects of nutrient loading rate, hydraulic retention time, and a substrate effectiveness for P sorption over time (objectives 2-3). Finally, the third article (Chapter 4) evaluates the effect of planting style and substrate on nutrient removal efficiency (Objectives 4-6)

CHAPTER 1: LITERATURE REVIEW

Non-point source runoff

Contaminants are exported from a variety of land uses including suburban, golf courses, agriculture, and forestry. One important class of contaminants is nutrients, including the many forms of inorganic and organic nitrogen and phosphorus. Nutrients are most often considered non-point pollutants but point source discharges, such as wastewater treatment plant effluent, may contribute significant quantities of nutrients to receiving surface waters. During the past 40 years we have begun to take action to reduce the loading of anthropogenic nutrients into the environment. Phosphorus was removed from products reducing overall use and other point source contributors were regulated. However, non-point source contributors are much harder to regulate and control. Various agricultural activities export nutrients during storm events. Quantities of exported nutrients usually depend on a variety of factors including application rates, crop production, soil type, and slope. Landscape nursery operations, particularly in warmer climates, rotate plants every 8 to 12 weeks resulting in extensive use of agrochemicals including nutrients. As water becomes increasingly regulated, these agricultural operations will have to manage their land to reduce, and even eliminate, nutrient export. While various strategies are being considered to treat runoff from landscape nursery operations, one strategy with considerable promise is the use of constructed wetlands..

Wetlands remove both point- and non-point source pollutants via four principal components – microbial colonies, the water column, emergent macrophytes, and the sediment/substrate (1). Emergent macrophytes contribute to the remediation and purification function of wetlands by providing a substrate for establishing microbial colonies. As water flow rates decrease because of stem density, sedimentation rates increase because of the slowed water flow. Oxygenation of sediments around plant roots increases via air transport through aerenchyma cells in hollow, fleshy stems. The sediment/substrate not only provides surfaces for growth of microbial colonies, it also supports the plant root system, and provides a variety of redox reactive surfaces for ion complexation. The water column is the link between surface water and the sediment and facilitates transport of chemicals and gases between microbial, plant, and animal communities.

Water quality varies seasonally with regard to concentrations of nitrogen (N), phosphorus (P), and dissolved oxygen (DO). Kawara et al. (2) noted that nutrient concentrations and chemical oxygen demand were low during winter and increased in the summer. Kröger et al. (3) noted internal wetland cycling during winter months resulted in increased export of nutrients from the decay of senesced plant material. Headley et al. (4) reported consistent background levels of internally generated organic nitrogen and phosphorus at 0.45 mg/L and 0.15 mg/L respectively. Several studies have shown that emergent plants rapidly leach nutrients into the water column when they begin to decompose (5-8). This rapid release of nutrients, especially phosphorus, during the least biologically active time, may allow large amounts of phosphorus to leave wetland areas and leach into nearby sensitive bodies of water.

Phosphorus: Background, chemistry, and sorption

Eutrophication

Manmade sources of P are commonly linked to stream and lake eutrophication. Phosphorus can be exported from erosion of agricultural land, agricultural runoff, urban runoff, as well as wastewater effluent (9). Eutrophication can be broadly defined as the process whereby water body productivity, both primary and secondary, increases in response to increased nutrient availability (10-11). Eutrophication is a naturally occurring process in surface waters, and a water body's progression from oligotrophic (< 5-10 μg/L of P and < 250-600 μg/L N), to mesotrophic (< 10-30 μg/L P and 500-1100 μg/L N), and finally to eutrophic depends on the normal nutrient status of the lake, its geography, watershed size and location, nutrient sources, and time (10). Typically, phosphorus is the most limiting nutrient for primary productivity in freshwater systems (12). Thus it is normally phosphorus that determines the rate at which natural eutrophication occurs (10).

Hyper-eutrophication occurs when excess P becomes available. Previous research has shown that P concentrations > 0.05 mg/L may contribute to increased eutrophication (13). Eutrophication itself is not an inherently unstable process, but water bodies with communities of organisms adapted to low nutrient concentrations (oligotrophic or mesotrophic water bodies) are more susceptible to overfertilization, while naturally eutrophic water bodies are more resilient to additional nutrients because their plant and animal communities are adapted to the more productive environment (10).

Non-point source phosphorus pollution is a problem because it becomes the driving force in the eutrophication process compromising the natural phosphorus cycle. When man-made sources increase the quantity of P reaching surface waters, the natural limit on primary productivity is removed, and growth can occur at an unsustainable rate. Algal blooms are a natural process in many water bodies, but the problem with excess P is that it facilitates additional algal growth, so that when the crash comes after the bloom there is more biomass containing large amounts of organic matter. Organic matter that is not incorporated into sediment is deposited along the shoreline and can change the physical habitat for littoral biota (both plant and animal). Particulate organic matter settling to sediments where bacterial processing occurs will increase the rate of processing leading to increased biological oxygen demand. The diminution of oxygen in the water column and sediment can have harmful impacts on organisms in deeper water and sediments (10, 14). If this decrease in oxygen occurs when the lake is highly stratified, the deeper portions of the lake may remain anoxic until turnover occurs. Thus, organisms used to higher oxygen concentrations and unable to adapt to the low oxygen or anoxic environment die. It is not the increase in nutrients that causes the death of the aquatic/benthic organisms; it is the secondary effect of the decrease in available oxygen.

Wetland cycle and chemistry

Phosphorus is a limiting nutrient in northern bogs, freshwater marshes, and southern deepwater swamps (15). In other wetlands, like agricultural wetlands, it is an important mineral, but not generally limiting due to its biochemical stability and relative abundance because of agricultural/non point source runoff. Wetland cycling of phosphorus involves its utilization in many pathways and various permanent and temporary sources and sinks (Figure 1; 16). Phosphorus is present as both soluble and insoluble complexes in wetland soils. The complexes are comprised of both organic and inorganic forms (Table 1). Orthophosphate or soluble reactive phosphorus is the principle inorganic form; its principle forms are PO_4^{-3}, HPO_4^{2-}, and $H_2PO_4^-$ and the predominant form present is based on wetland pH (Figure 2). Soluble reactive phosphorus is the analytical measure of orthophosphate that is biologically available, although the two terms are not exactly equivalent (10). Both insoluble organic and inorganic forms of phosphorus and dissolved organic phosphorus are not biologically available until their transformation into soluble inorganic P forms.

Phosphorus is only indirectly affected in sediments by changes in redox potential. This indirect change in P form is induced by association with elements whose forms are influenced by changes in redox potential (elements such as Fe and Mn). Phosphorus availability to plants is reduced under aerobic conditions when insoluble phosphates precipitate with calcium carbonate, aluminum, or ferric iron (Fe^{3+} oxyhydroxide; 16). Phosphorus also becomes less available after binding to organic matter as a result of incorporation into living biomass, or after it sorbs onto ferric and aluminum hydroxides and oxides, organic peat, or clay particles. The chemical bonding of the positively charged edges of the clay with negatively charged phosphates and the substitution of silicate by phosphate in the clay particle are both processes that contribute to P sorption (17).

Under anaerobic conditions, ferric iron is reduced to a more soluble ferrous (Fe^{2+}) compound; phosphorus bound to ferric iron is then released to solution when ferric iron is transformed to ferrous. Changing pH can also promote release of phosphorus from insoluble salts, either due to production of nitric or sulfuric acids or organic acids by chemosynthetic bacteria (15). Slightly acidic to acidic conditions may promote P sorption onto clay particles (17). The complexity of phosphorus mineral chemistry makes quantitative solubility calculations difficult, but some solubility trends can be discussed. The first trend is noticeable in acid conditions and dependent upon iron and aluminum availability. Under this condition, phosphorus may be fixed and insoluble. The second trend involves fixation of phosphorus by calcium and magnesium when environmental conditions are alkaline. The final trend involves the solubilization of iron minerals and subsequent release of phosphorus from them when conditions are reducing/anaerobic. Under reducing conditions the presence of sulfide can shift the balance from phosphorus:iron complexes to the formation of iron sulfide, which can prevent phosphorus mineralization with iron (16).

Sorption and desorption mechanisms and characterization

The most straightforward way to describe sorption is to plot the amount of phosphorus sorbed (sorbate) as a function of the amount of phosphorus available in solution, after an equilibration period at a steady temperature (18-19). Typically the highest amount of phosphorus is sorbed at the lowest solution phosphorus concentrations, or in other words, the lower the phosphorus concentration available in the water column, the greater the proportion of phosphorus sorbed to available solids. At high phosphorus concentrations a smaller proportion of phosphorus is actually sorbed to available binding sites. If concentrations of sorbate are high enough, all binding sites will be saturated and no further sorption can occur.

When phosphorus sorption isotherms are performed, cation balancing solutions are always used. The cation balance solution, whether $CaCl_2$, KCl, or NaCl, enhances phosphorus sorption rate to media (20-21). The cation species used in the ionic balance solution influences the rate at which equilibrium between the solution and media is reached. If a fast sorption reaction is desired a divalent cation (like Ca^{2+}) should be used, but if higher concentrations of phosphorus are being tested, a monovalent cation (like K^+ or Na^+) should be used to minimize precipitation of insoluble calcium phosphates. The ionic strength of the solution also influences the rate of sorption. Higher ionic strength solutions, typically ranging between 0.01 to 0.1 M, reduce the thickness of the diffusion layer and thus maintain a larger concentration of phosphorus ions near the media surface which enhances initial phosphorus sorption (20).

The simplest models used to describe the relationship between concentration and amount sorbed are termed isotherm equations (19). The Freundlich equation is the simplest of these and assumes an infinite supply of unsaturated binding sites and may be linearized by taking the log of the original equation:

$$\log S = b \log C_T + \log K \qquad [1]$$

where, S = amount of added sorbate retained by media (mg/kg), K and *b* are constants, and C_T = the aqueous concentration (mg/L) after 24 hour equilibration (22). The

Freundlich model assumptions are met if the data is linearized by this transformation. If the data is linear, the intercept = K and the slope = *b*.

The Langmuir isotherm assumes a three-dimensional binding surface with a finite phosphorus sorption capacity limited by the number of internal and external binding sites. It can be linearized using the following inversion of the original equation:

$$\frac{C_T}{S} = \left(\frac{1}{k * S_{max}}\right) + \left(\frac{C_T}{S_{max}}\right) \qquad [2]$$

where, S_{max} is the maximum possible sorption by the solid in mg/kg, representing a monolayer surface coverage, and is the value of C_T/S where the curve flattens. The intercept is equal to $1/k*S_{max}$ and the slope is equal to $1/S_{max}$ (19, 23-24).

Desorption occurs if the phosphorus (sorbate) is reversibly bound to the media surface. The phosphorus retention capacity (P_r) of a media is defined as the amount of phosphorus irreversibly bound to media after 24 h. It is determined by calculating the difference between the amount of phosphorus adsorbed (P_{ad}) to media and the amount of phosphorus recovered from (or desorbed from) the media after a 24h desorption period in an ionic solution with no additional phosphorus. The formula to describe this retention relationship is as follows:

$$P_r = P_{ad} * f \qquad [3]$$

where, f = the mean fraction of phosphorus retained by the media and is derived from P_r/P_{ad} (23). Thus to calculate the proportion of phosphorus retained by the media after a specific desorption period, utilize P_r = f*100 (23).

Many media have been incorporated into subsurface-flow wetlands because of their phosphorus sorption capacities including, gravel, dolomite, furnace slag, fly ash, shale, limestone, and sand (23, 25-27). These media have improved the phosphorus retention capacity of constructed wetlands. Many of these materials are inexpensive and naturally occurring and can be used as the root bed media for subsurface-flow constructed wetland systems. When choosing a media to incorporate into subsurface flow wetlands it is important to evaluate its utility and its relative availability. Is it a recycled product or does it require mining or harvesting to be useable? Can we find a material that is recycled or could be recycled after its initial use? Short-term monetary savings alone should not drive the decision making process, instead both short- and long-term costs should be evaluated.

Constructed Wetlands

Wetlands assimilate and transform nutrients via a range of physical and biological mechanisms (1, 3, 23). One such mechanism is plant uptake, but it accounts for < 5% of nutrient removal from contaminated waters (1). Only in those systems with low nutrient loading rates, i.e. vegetative ditches and drainage canals, does plant uptake become a primary nutrient removal mechanism (3). Constructed wetlands function similarly to natural wetlands and can be built to target specific remediation goals.

The majority of constructed wetlands used for wastewater treatment are surface-flow or free water surface systems (1). The flow pattern of surface-flow systems directs water flow above media surfaces, which are typically clay or native soil. In subsurface-flow constructed wetlands water flows directly through media and is not generally visible. A drawback to the use of subsurface-flow wetlands is their tendency to clog after less than two years of heavy operation, e.g., sewage and dairy effluent, even though the filler media utilized is generally gravel or crushed rock. Thus, surface-flow systems comprise the majority of treatment constructed wetland systems in the U.S., when any function less than tertiary polishing is needed.

Over the past 30 years, subsurface-flow wetlands have been used to treat nutrient rich wastewaters (4) and have been shown very effective at nutrient removal, especially for nitrate. However, performance efficiencies vary, ranging from 20 to 95%. Subsurface-flow systems also seem to be ideal for phosphorus remediation if the root bed media used for fill has been chosen based on its capacity for phosphorus sorption (23). The long-term viability of the root bed media is based on the contaminant loading rate and desired effluent quality.

Constructed wetlands are typically built so gravity flow directs water movement between cells, so that water pumping between treatment cells is unnecessary (1). Constructed wetland size is determined by both the nutrient (or contaminant) loading rate and by the daily volume of wastewater that must be treated. If the nutrient-loading rate is high, the surface area of the constructed wetland system needed for treatment may increase in order to provide the needed detention time for nutrient transformation/uptake. To reduce excessive loading into the front of the wetland cells, they should be configured so that individual cells have a 2-5:1 length to width ratio (1, 16). This configuration guideline helps to prevent short-circuiting and allows for more even distribution of the inflow water.

The quantity and relative dispersion of nutrient contaminants comprising non-point source pollution makes treatment via conventional means (wastewater treatment plants) untenable (28). Instead, both natural and constructed wetlands are being used as tools to help remove nutrient contaminants introduced from non-point source locations. The general efficiency with which a natural wetland removes nutrients varies from wetland to wetland, and many water quality factors influence wetland responses making them unpredictable and non-linear (28). However, constructed wetland design can be optimized so that consistent nutrient removal efficiencies can be achieved.

Mesocosms

Aquatic and terrestrial ecosystems are generally studied through the use of research mesocosms, first because of their statistical power and second for their ease of replication (29). Mesocosms have been used to evaluate commercial scale applications, such as wastewater treatment and food production, and for ecological engineering and ecosystem restoration projects. Over the last 25 years, mesocosms have been used in wetland science for studies examining pollutant fate and effects, biogeochemical cycling, and ecosystem dynamics as impacted by nutrients. Mesocosms facilitate relevant research that is replicated, repeatable, and less costly than larger, field-scale studies.

However, mesocosms are somewhat limited in scope, because they cannot always reflect the complex interactions that occur in natural ecosystems. Mesocosms are very useful for explaining ecosystem processes when measured over a short time, but a mesocosm should not be used to study biogeochemical processes for more than two years, due to increasingly pot-bound vegetation and redox conditions that become reducing more quickly than would normally occur in a field-scale constructed wetland. Differences in complexity between a mesocosm and a large-scale wetland can dramatically impact biogeochemical functions and pathways. This loss of complexity with mesocosms makes it difficult to simulate accurately all of the physical and biological conditions and their interactions, for example the effects of sediment-water interface turbulence, water mixing, and boundaries (i.e. walls). Even though the loss of ecological complexity may occur, depending upon mesocosm size, the advantages of their use, namely replicability, lower costs, and improved statistical power, increase these 'model systems' frequency of use. Ecosystem management decisions should not be solely based on mesocosm studies; instead some ecosystem-scale studies should be conducted for full confidence in management decisions (29).

Hydrology

The study by Ahn and Mitsch (29) compared a large wetland and ten mesocosms with similar hydraulic loading rates (HLRs) and hydraulic retention times (HRTs). They noted it was possible to overestimate the retention time of the large wetland because of the assumption that the entire volume of water is involved in the flow. This is not always the case since the water mixing differs between the two-wetland scales. In large-scale wetland systems basin morphology and topography may contribute to reduced mixing and thus to shorter retention times. Kadlec and Knight (16) noted that retention time is a critical factor controlling nutrient retention in wetlands.

Physiochemistry

Ahn and Mitsch (29) found their larger wetland showed consistent water temperature increases from inflow to outflow over the four-year study; while, mesocosm water temperatures decreased over the two-year period. Dissolved oxygen concentrations also differed when comparing the large wetland, where dissolved oxygen increased for two of the four study years, whereas with the mesocosms, the second study year showed a 50% decrease. The anaerobic conditions of the wetland sediments led to a yearly decrease in water redox potential in both the large and mesocosm wetlands.

Ahn and Mitsch (29) suggest that a normal coefficient of variation (CV = (standard deviation/mean) x 100]) range for mesocosm variables simulating a large field system varies from 20 to 30%. If a mesocosm has high variability it may fail to adequately simulate a large-scale wetland. They noted similar variability between the mesocosm (CV = 16% on average) and large (CV = 20% on average) wetlands with regard to inflow water chemistry parameters: temperature, DO, pH, conductivity, and redox potential.

Nutrient cycling

Phosphorus may be removed from the water column in wetlands via several biologically and physically mediated mechanisms including plant uptake, accretion into wetland soils, immobilization by microbes, sorption to root bed media, and precipitation (23, 30). To ultimately remove excess phosphorus from wetland cycling, both plant harvest (to remove plant material before it decays and leaches phosphorus) and removal of saturated root bed media are needed. Periodic plant harvesting seems a viable solution, but experiments performed by Kim and Geary (31) found little decrease in total phosphorus in water column when comparing cells with harvested plants and treatment cells where no harvesting occurred. They attributed the small change in phosphorus remediation efficiencies to the energy required for plant regrowth to begin and time taken for plants to regrow after harvesting occurred. Phosphorus desorption from root bed media can occur under both aerobic and anaerobic conditions. Phosphorus is also released into the water column as plant material senesces and undergoes decomposition (3). Desorption and decomposition are both potential sources of internal wetland loading of phosphorus. Ahn and Mitsch (29) noted that phosphorus was exported from their mesocosms during the second year of their study after dissolved oxygen and redox potential dropped significantly.

The work by Kröger et al. (3) with *Leersia oryzoides* examined its capacity for luxury uptake of phosphorus and nitrogen and the difference in nutrient loss during senescence from plants provided with normal or excess nutrient levels. During the first week of plant senescence, *L. oryzoides* plants with excess nutrients exported > 3.5 mg/L nitrogen (NH_3 and NO_3) and > 3.0 mg/L phosphorus into the water column. By contrast, plants supplied with normal nutrient levels exported similar nitrogen concentrations but phosphorus concentrations were below 1 mg/L. However, after the first week, nitrogen release into the water column was reduced to less than 0.25 mg/L, but phosphorus export from both enriched (~ 3.0 mg/L) and non-enriched (~ 0.75 mg/L) plants remained at similar concentrations throughout the 12 weeks of sampling.

Headley et al. (32) found that 96% of the influent phosphorus load was consistently removed by reed beds (*Phragmites australis*), with total phosphorus concentrations reduced from 0.5 mg·L^{-1} to 0.005 mg·L^{-1} without regard to loading rate or season studied. They also identified three seasonal stages in the uptake and cycling of phosphorus by *P. australis* during the second and third years. The first is a period of rapid aboveground growth during the spring fueled partially by rhizome-accumulated phosphorus reserves. Then, during summer, the influent phosphorus loading rate governed the aboveground biomass uptake, while the belowground biomass phosphorus uptake remained relatively stable. Finally, during the fall and winter, P appeared to be translocated to reserves in the rhizomes from senescent shoots. They found 85% of the below-ground biomass P was in the top 20 cm of the substrate and that gravel P fixation increased in importance from 12% for the first year to approximately 30% of the P removal in the second year. There was a correlation between P fixed by gravel and influent P loading rate. Most of the P bound to the gravel was weakly bound in the lower 30 cm of substrate during the second year. Phosphorus incorporation into detritus, microbiota, or other compartments increased after the first year and became one of the most important P removal processes. Consequently, while plant uptake occurred and phosphorus was stored in *P. australis* root systems, the controlling factor for phosphorus removal was not biological removal but rather the physical and chemical reactions that took place in the wetland ecosystem.

Vegetation

Plants uptake phosphorus in its highest oxidized form ($H_2PO_4^-$), and unlike nitrogen or sulfur, phosphorus remains in this form and is not reduced. After uptake, the phosphorus form at physiological pH is generally $H_2PO_4^-$. Phosphorus either remains in its inorganic form, is esterified to a simple sugar phosphate, or becomes part of high-energy pyrophosphate bonds. The rates of exchange between these three forms are very high in plants, and are dependent upon their location within the plant (33). The phosphorus required by a plant for optimal growth is between 0.3 and 0.5% by mass during vegetative growth. Plants regulate the amount of phosphorus they allow to enter through their root cells. When plants that have undergone phosphorus deficient conditions become exposed to adequate phosphorus, they will initially (only for a few hours) take up higher concentrations of phosphorus and translocate it into their shoots. Once a certain internal phosphorus concentration has been attained, internal feedback mechanisms decrease the amount of phosphorus able to enter through the roots. Thus, luxury phosphorus uptake is not a viable expectation for most plant species because they do not have the capacity to take up more phosphorus than they physiologically need (33).

Ahn and Mitsch (29) determined two factors altered macrophyte productivity. The first was a scale artifact observed from macrophyte overhang. Macrophyte overhang involves sunlight reaching a larger proportion of plant stems than that of typical wetlands, due to the higher area/edge ratio of a mesocosm. The second scale artifact is seen in the vegetation spatial pattern; in natural wetlands rarely does vegetation cover 100% of the surface area. This is due to beaver, goose, and muskrat herbivory, harvesting, depth variation, and other disturbances; while mesocosms often have 100% vegetative cover, simply due to their smaller size. These two factors contribute to complete water column shading and increased wind protection. Thus, when mesocosms are compared with large-area wetlands, mesocosms exhibit cooler water temperatures, lower redox potentials, higher conductivity, and lower DO, all impacting and changing mesocosm nutrient uptake when compared with larger-scale wetlands. Wetland function is not controlled by nutrient inflow and hydrology alone, but also by stream damming, transpiration, sediment excavation, detrital buildup, etc., all part of biotic feedback effects (15).

References

(1) Hammer, D.A. Designing contrasted wetland systems to treat agricultural nonpoint source pollution. *Ecol. Eng.* 1992, *1*(1), 49-82.

(2) Kawara, O., Uehara, M., Ibaragi, K. A study on the water quality of runoff from forest. *Wat. Sci. Tech.* 1999, *39*(12), 93-98.

(3) Kröger, R., Holland, M.M., Moore, M.T., Cooper, C.M. Plant senescence: A mechanism for nutrient release in temperate agricultural wetlands. *Env. Pol.* 2007, *146*, 114-119.

(4) Headley, T.R., Huett, D.O., Davison, L. The removal of nutrients from plant nursery irrigation runoff in subsurface horizontal-flow wetlands. *Wat. Sci. Tech.* 2001, *44*(11-12), 77-84.

(5) Gaudet, J.J. Uptake, accumulation, and loss of nutrients by papyrus in tropical swamps. *Ecol.* 1977, *58*(2), 415-422.

(6) Johnston, C.A. Sediment and nutrient retention by freshwater wetlands: Effects on surface water quality. *Crit. Rev. Env. Control.* 1991, *21*(5-6), 491-565.

(7) Keuhn, K.A., Gessner, M.O., Wetzel, R.G., Suberkropp, K. Decomposition and CO_2 evolution from standing litter of the emergent macrophyte *Erianthus giganteus. Micro, Ecol.* 1999, *38*(1), 50-57.

(8) Keuhn, K.A., Suberkropp, K. Decomposition of standing litter of the freshwater emergent macrophyte *Juncus effusus*. *Freshwat. Biol.* 1998, *40*(4), 717-727.

(9) Brooks, A.S., Rozenwald, M.N., Geohring, L.D., Lion, L.W., Steenhuis, T.S. Phosphorus removal by wollastonite: a constructed wetland substrate. *Ecol. Eng.* 2000, *15*, 121-132.

(10) Khan, F.A., Ansari, A.A. Eutrophication: An ecological vision. *Botan. Rev.* 2005, *71*(4), 449-482.

(11) Wight, D.A., Welbourn, P. *Environmental Toxicology*. Cambridge environmental chemistry series. Cambridge University Press. 2002, pp. 249-315.

(12) Conley, D.J. 2000. Biogeochemical nutrient cycles and nutrient management strategies. *Hydrobiol.* 2000, *410*, 87-96.

(13) Hinesly, R.L., Jones, T.D. Phosphorus in waters from sewage sludge amended lysimeters. *Env. Pol.* 1990, *65*, 293-309.

(14) USEPA. *Nutrient Criteria Technical Guidance Manual*. Office of Water and Office of Science and Science and Technology. EPA-822-R-07-004. 2007.

(15) Mitsch, W.J., Gosselink, J.G. *Wetlands*. Van Nostrand Reinhold, New York, N.Y. 1986. p. 539.

(16) Kadlec, R., Knight, R. *Treatment Wetlands*. CRC Press, Boca Raton, FL. 1996. p. 893.

(17) Stumm, W., J.J. Morgan. *Aquatic Chemistry*. 3rd ed. Wiley-Interscience. 1996. New York.

(18) Fox, R.L., Kamprath, E.J. Phosphate sorption isotherms for evaluating the phosphate requirements of soils. *Soil Sci. Soci. Am. Proc.* 1970, *34*, 902-906.

(19) Langmuir, D. *Aqueous Environmental Geochemistry*. Prentice Hall. Upper Saddle River, NJ. 1997.

(20) Ryden, J.C., Syers, J.K. Rationalization of ionic strength and cation effects on phosphate sorption by soils. *J. Soil Sci.* 1975, *24*(4), 395-406.

(21) Barrow, N.J., Shaw, T.C. Effects of ionic strength and nature of the cation on desorption of phosphate from soil. *J. Soil Sci.* 1979, *30*, 53-65.

(22) Casey, R.E., Taylor, M.D., Klaine, S.J. Mechanisms of nutrient attenuation in a subsurface flow riparian wetland. *J.Env. Qual.* 2001, *30*(5), 1732-1737.

(23) Pant, H.K., Reddy, K.R., Lemon, E. Phosphorus retention capacity of root bed media of sub-surface flow constructed wetlands. *Ecol. Eng.* 2001, *17*, 345-355.

(24) Woodruff, J.R., Kamprath, E.J. Phosphorus adsorption maximum as measured by Langmuir isotherm and its relationship to phosphorus availability. *Soil Sci. Soc. Amer. Proc.* 1965, *29*(2),148-150.

(25) Prochaska, C.A., Zouboulis, A.I. Removal of phosphates by pilot vertical-flow constructed wetlands using a mixture of sand and dolomite as a substrate. *Ecological Engineering.* 2006, *26*, 293-303.

(26) Vohla, C., Alas, R., Nurk, K., Baatz, S., Mander, Ü. Dynamics of phosphorus, nitrogen and carbon removal in a horizontal subsurface flow constructed wetland. *Sci. Tot. Env.* 2007, *380*, 66-74.

(27) Xu, D., Xu, J., Wu, J., Muhammad, A. Studies on the phosphorus sorption capacity of substrates used in constructed wetland systems. *Chemos.* 2006, *63*, 344-352.

(28) Gumbricht, T. Nutrient removal processes in freshwater submersed macrophyte systems. *Ecol. Eng.* 1993, *2*, 1-30.

(29) Ahn, C., Mitsch, W.J. Scaling considerations of mesocosm wetlands in simulating large created freshwater marshes. *Ecological Engineering.* 2002, *18*(3), 327-342.

(30) Richardson, C.J. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Sci.* 1985, *228*(4706), 1424-1427.

(31) Kim, S.Y., Geary, P.M. 2001. The impact of biomass harvesting on phosphorus uptake by wetland plants. *Water Science and Technology.* 2001, *144*(11-12), 61-67.

(32) Headley, T.R., Huett, D.O., Davison, L. Seasonal variation in phosphorus removal processes within reed beds – mass balance investigations. *Wat. Sci.Tech.* 2003, *48*(5), 59-66.

(33) Marschner, H. *Mineral Nutrition of Higher Plants.* 2nd ed. Academic Press Ltd., Cambridge, UK. 1995.

(34) Brady, N.C., and R.R. Weil. *The Nature and Property of Soils.* 12th ed. Prentice Hall, London. 1990.

Figure 1.1. Phosphorus biogeochemical cycle (15-16, 34).

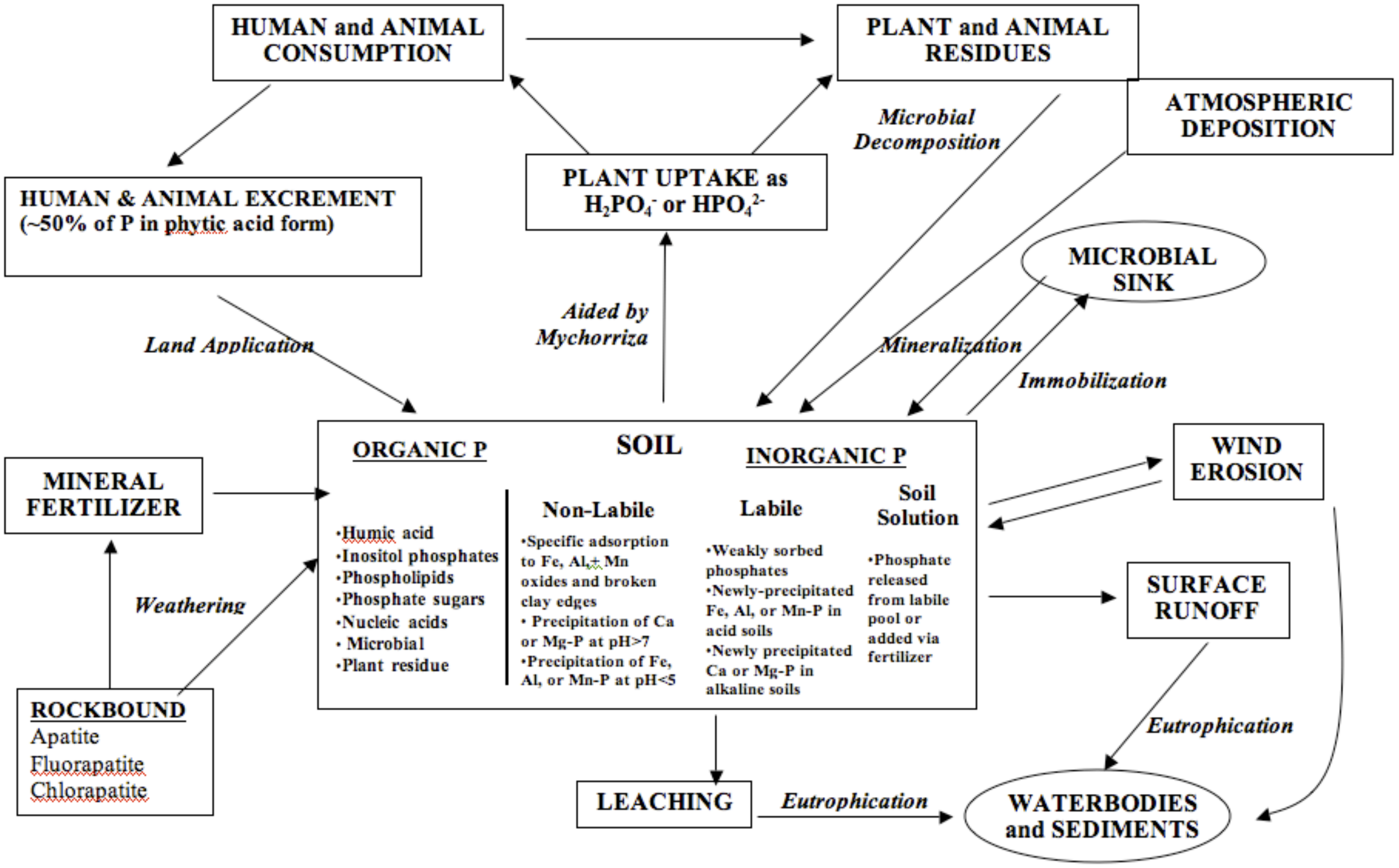

Figure 1.2. Distribution of phosphorus species expressed as a fraction as a function of aqueous solution pH (34).

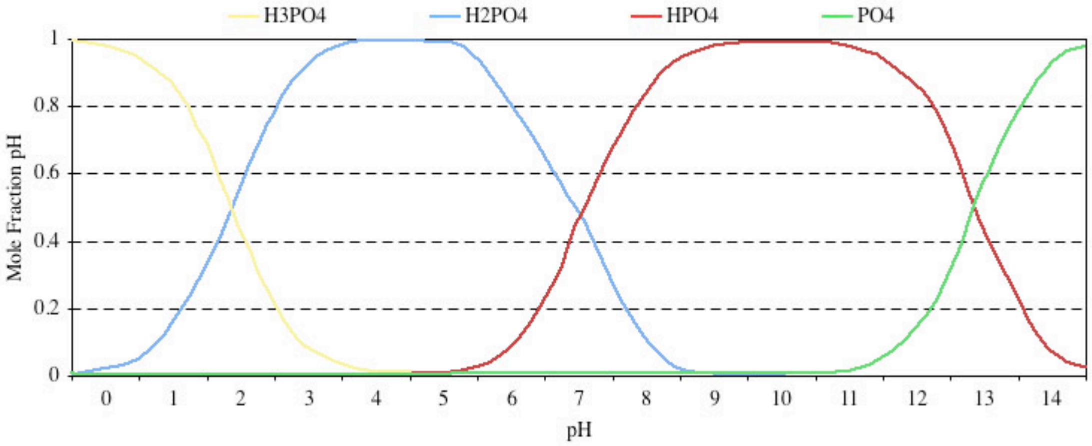

Table 1.1. Major types of dissolved and insoluble phosphorus in the wetland environment (15-16).

Phosphorus	*Soluble Forms*	*Insoluble Forms and Precipitates*
Inorganic	orthophosphates ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-})	clay-phosphate complexes
	ferric phosphate ($FeHPO_4^+$)	metal-hydroxide phosphate apatite ($Ca_5(Cl,F)(PO_4)$)
	soluble reactive phosphorus (PO_4-P)	hydroxylapatite ($Ca_5(OH)(PO_4)_3$ variscite $Al(PO_4)\cdot 2H_2O$
	condensed phosphates (pyro-,meta-, and poly-phosphates)	stringite $Fe(H_2PO_4)\cdot 2H_2O$ vivianite $Fe_3(PO_4)_2\cdot 8H_2O$ wavellite $Al_3(OH_3)(PO_4)_2\cdot 5H_2O$
	phosphine gas (PH_3)	
Organic	dissolved organics, e.g., sugar phosphates, inositol phosphates, phospholipids, phosphoproteins	insoluble organic phosphorus bound in organic matter

CHAPTER 2: BRICK AND INDUSTRIAL MINERAL AGGREGATE: PHOSPHORUS SORBING MEDIA FOR CONSTRUCTED WETLAND SYSTEMS

Abstract

Constructed wetland systems built to handle nutrient contaminants are often efficient at removing nitrogen, but ineffective at reducing phosphorus (P) loads. The incorporation of media with large phosphorus sorption capacities often improves phosphorus removal in subsurface-flow constructed wetland systems. Crushed brick, a recycled building product, and a palygorskite-bentonite industrial mineral aggregate (calcined clay) were screened for their P sorbing capacities using sorption isotherms. Also, their P retention capacities were examined with a series of desorption experiments. The fine calcined clay had the highest average P sorption capacity (1239.5 mg kg^{-1}). The coarse calcined clay average sorption capacity was 497.1 mg kg^{-1}, and the average P sorbed by brick was 102.4 mg kg^{-1}. The coarse clay sorbed the highest percentage of P supplied (76%), except at exposure concentrations > 500 mg l^{-1} where the increased surface area of the fine calcined clay augmented its P sorption capacity enabling it to sorb a slightly greater percentage of P from solution. The three substrates bound the P tightly, and desorbed < 3% (brick) to < 1% (fine calcined clay) of the P initially sorbed. The calcined clays exhibited greater P sorption capacities than the brick. Thus, a constructed wetland system established with calcined clay would have a longer expected lifetime than

a system established with brick. Estimates of substrate life spans are highly influenced by P load and the daily flow rate into the constructed wetland system.

Keywords

Bentonite, Palygorskite, Langmuir isotherm, Freundlich isotherm, Sorption, Desorption

Introduction

Nutrient contamination in surface waters is garnering increased attention both locally and globally because of its potential for deleterious impacts to humans and the environment (1-2). Scrutiny of local, state, and federal environmental agencies as a result of increasing concerns about water quality preservation and improvement is likely to lead to formation of new water quality criteria for previously unregulated agricultural non-point source contributors (3-4). Constructed wetland systems (CWS) are useful for reducing nitrogen (N), phosphorus (P), biological oxygen demand, and suspended solids (5-7).

The phosphorus remediation efficiencies of CWS still often result in export concentrations greater than 0.05 mg l^{-1} P, which has been shown to contribute to increased eutrophication (8-10). Phosphorus is not as consistently managed via CWS because internal phosphorus cycling often leads to the export of phosphorus (3-4, 11). Of the various P immobilization pathways in CWS, fixation by substrate is the most reliable, and thus the factor that could be most easily manipulated for control (6). Phosphorus sorption efficiencies of many substrates have been examined including: alunite, gravel, dolomite, furnace slag, fly ash, shale, limestone, and sand (7, 12-15), and their relative P sorption capacities characterized. Sorption of phosphate by clays has interested many researchers because of their positive surface charge, high ion-exchange capacities, and high affinity for P (16-17). The upper limit for current-day phosphorus remediation strategies is thought to be 2,060 mg P kg^{-1} soil for amorphous, composite materials containing Fe, Al, oxides, and kaolinite (18).

When choosing a substrate for P removal, several characteristics and properties are desirable. The substrate should exhibit, high selectivity and a large capacity for P, handling ease (cannot be too fine or coarse), high physical and chemical strength, and be nonhazardous (16). An additional benefit would be a substrate that is cost effective, available from sustainable sources, and can be recycled or reused. Reuse of the substrate is especially important because once a substrate becomes P saturated, it could serve as a potential fertilizer source in place of or in addition to traditional fertilizers, if desorption from the media occurs gradually over time at sufficient concentrations. The objectives of this study were to characterize the sorption capacities of three clay media and relate this to their effectiveness as substrates for CWS, evaluate their desorption rates and concentrations, and determine the equilibration period necessary for maximal P sorption.

Methods

Industrial mineral aggregate

The palygorskite-bentonite mineral aggregate (calcined clay, CC) evaluated was mined in Ochlocknee, GA (Oil-dri Corp. of America, Chicago). Two particle sizes were examined, a coarse 0.8 to 4.75 mm (4/20 LVM) and a fine 0.25 to 0.85 mm (24/48 LVM). Both had been pretreated to a temperature of 800 °C.

Brick

The brick, a manufacturing waste by-product, was examined at only one range of particle sizes, 0.8 to 4.57 mm (4/20, National Brick Research Institute, Clemson, SC). The brick manufacturing process involved firing the clay, and a range of temperatures (149 to 982 ºC) was used depending upon the type of clay in the brick (19). Since this brick is a waste product, the maximum temperature to which it was heated was not known.

Phosphorus sorption experiments

Phosphorus sorption experiments were conducted with brick and fine and coarse CC using a batch incubation technique under anaerobic conditions. Approximately 2 g of the fine and coarse CC were placed in 50 ml centrifuge tubes and treated with 45 ml of 0.01 M $CaCl_2$ solution containing 0, 0.5, 1.0, 10.0, 50.0, 100.0, 500.0, or 1000.0 mg l^{-1} P provided as KH_2PO_4. Six replicates per media type and exposure concentration were prepared and allowed to equilibrate for 24 h at 25 ± 2 ºC, on a rotary shaker table set at 150 rpm. Equilibrated samples were centrifuged at 3500 rpm for 15 min and filtered through 0.45 μm polytetrafluoroethylene (PTFE) filters. Total soluble P was measured in the filtrate using ICP MS/OES.

Approximately 3 g of brick were placed into 50 ml centrifuge tubes and treated with 45 ml of 0.01 M KCl solution containing 0, 0.5, 1.0, 10.0, 50.0, 100.0, 250.0, or 1000.0 mg l^{-1}P provided as KH_2PO_4. Six replicates per exposure concentration were used. The samples were equilibrated for 24 h at 25 ± 2 ºC on a rotary shaker table set at 150 rpm. Equilibrated samples were centrifuged at 3500 rpm for 15 min and filtered through 0.45 μm PTFE filters. Total soluble P was measured in the filtrate using ICP MS/OES. Phosphorus not recovered in the filtrate of the three substrates was considered sorbed to substrate.

Phosphorus desorption experiments

Phosphorus desorption experiments were performed immediately on substrate residues from sorption experiments. The CC residues were equilibrated with 45 ml of 0.01 M $CaCl_2$ solutions for 24 and 48 h at 25 ± 2 ºC. Equilibrated samples were centrifuged for 15 min at 3500 rpm and filtered through 0.45 μm PTFE filters. The filtrates were analyzed for soluble reactive phosphorus using a Dionex AS50 IC. The brick residues were equilibrated with 45 ml of 0.01 M KCl solutions for 24 and 48 h at 25 ± 2 ºC and were handled in the same manner as the CC residues. The amount of P retained by the substrate was calculated from the difference in the amount of P sorbed to the media and the amount recovered from solution.

Substrate equilibration period

Approximately 2 g of fine and coarse CC were equilibrated with 45 ml of 0.01 M KCl solution containing 500 mg P l^{-1} for 0, 12, 24, 48, 72, 96, 120, 144, 168, or 192 h at 25 ± 2 ºC. Approximately 2 g of brick were equilibrated with 45 ml of 0.01 M KCl solution containing 250 mg P l^{-1} for the same time points. There were six replicates per time period for both CC and brick and samples were handled as above. Phosphorus not recovered in the filtrate of the three substrates screened was considered sorbed to substrate.

Sorption characterization

At low P concentrations the relationship between P added and P sorbed is generally linear (12). Sorption data were fit to Freundlich and Langmuir models. The Freundlich equation is the simplest and assumes an infinite supply of unsaturated binding sites. The Freundlich equation may be linearized by the following log transformation:

$$\log S \;=\; b \log C_T \;+\; \log K \qquad [1]$$

where, S = amount of added sorbate retained by media (mg kg^{-1}), K and *b* are constants, and C_T = the aqueous concentration (mg l^{-1}) after 24 hour equilibration. The Freundlich model assumptions are met if the data are linearized by this transformation. If the data are linear, the intercept = logK and the slope = *b*.

The Langmuir isotherm assumes a three-dimensional binding surface with a finite phosphorus sorption capacity limited by number of internal and external binding sites. It can be linearized using the following inversion of the original equation:

$$\frac{C_T}{S} = \left(\frac{1}{k * S_{max}}\right) + \left(\frac{C_T}{S_{max}}\right) \qquad [2]$$

where S_{max} is the maximum possible P sorption by the solid (mg kg^{-1}) and equals the value of $\frac{C_T}{S}$ where the fitted curve flattens. The intercept is $\frac{1}{k * S_{max}}$, and the slope is equal to $\frac{1}{S_{max}}$ (12, 20-21).

Removal efficiencies were also calculated using:

$$\text{Removal efficiency (\%)} = \frac{\left(C_I - C_T\right)}{C_I} \text{ x } 100 \qquad [3]$$

where C_I = initial exposure concentration and C_T = aqueous concentration after 24 h equilibration period.

Desorption characterization

The substrate phosphorus retention capacity (P_R) was defined as the amount of P bound to media after 24 h or 48 h. The difference between the amount of phosphorus adsorbed (P_{ad}) to media and the amount of phosphorus recovered from (or desorbed from) the media after a 24h and 48 h desorption period in an ionic solution containing no additional phosphorus was calculated. The formula to describe this retention relationship is as follows:

$$P_R = P_{ad} * f \qquad [4]$$

where f = the mean fraction of phosphorus retained by the media. Following the methods of Pant et al. (12) P_R vs. P_{ad} for each substrate was plotted and the intercept was forced through the origin. The slope obtained by graphing this relationship correlates with the calculated parameter f.

Data analyses

Data were fit to isotherm models and statistical analyses were performed on transformed data using SAS PROC REG (SAS Institute Inc. Cary, NC) for homogeneity of slopes and intercepts. Treatment comparisons were made using SAS PROC GLM with LSD mean comparisons when appropriate ($\alpha = 0.05$).

Results and discussion

Substrate P sorption

Substrate P sorption capacity was examined by linearizing data using both the Freundlich and Langmuir isotherm models (Fig. 2.1). Model parameters were then examined to determine the model that best illustrated sorption trends (Table 2.1). The Freundlich model best described the linear brick sorption ($r^2 = 0.957$; Fig 2.1A). The Langmuir model best illustrated the P sorption relationship with both fine ($r^2 = 0.940$; Fig 2.1B) and coarse ($r^2 = 0.934$) CCs. The P sorption capacity of the CCs was much greater than the brick (Fig. 2.1 and Table 2.1). The adsorption maxima for the coarse and fine CCs as described by the Langmuir S_{max} were 256.28 mg kg^{-1} and 462.85 mg kg^{-1}, respectively. A comparison of the Langmuir *k* values, which illustrate the relative binding strength of each media for P (12), showed that coarse CC had the greatest binding strength and brick the lowest.

When P exposure concentrations were between 1.0 mg l^{-1} and 500.0 mg l^{-1}, fine CC sorbed P more strongly than the coarse CC, which sorbed P more strongly than did the brick ($P < 0.0001$). However, when P exposure concentrations were < 1.0 mg l^{-1} the coarse and fine CC sorbed P in a similar manner, although both sorbed P more strongly than the brick ($P < 0.0001$). Homogeneity of slope analyses using SAS PROC REG indicated the P sorption rate to fine and coarse CC was different from P sorption rate to brick ($P < 0.0001$). Brick sorbed P at a faster rate ($b = 0.741$) than both fine and coarse CCs ($b = 0.390$ and $b = 0.301$, respectively). All substrates examined had a $b < 1.0$ which suggests saturation is possible and sorption processes will be limited by binding site availability (8). Relative media sorption capacities were greater for the CCs than the brick, indicating that even with slower sorption rates, CCs were able to sorb more P.

Homogeneity of intercept analyses compared the linear model intercepts. The brick intercept differed significantly from the intercepts of both CCs ($P < 0.0001$). Further exploring this relationship, Fig. 2.2 depicts the removal efficiency of each media at specific exposure concentrations. The coarse CC was generally the most efficient at P sorption when exposure concentrations were ≤ 100 mg L^{-1} P, with removal efficiencies ranging from 13 – 98 %. The brick had consistently lower removal efficiencies with a range of 5 – 51% for 0.5 – 100 mg l^{-1} P equilibration solutions. The fine calcined clay generally had higher removal efficiencies than the brick, but lower removal efficiencies than the coarse CC.

The coarse CC capacity for P sorption (S_{max} = 256.3 mg kg^{-1}) and its higher binding strength (k = 9.79) indicated its effectiveness at P sorption when P exposure concentrations were between 0.5 and 100 mg L^{-1}. The binding sites of the coarse CC were saturated after exposure to the 500 and 1000 mg l^{-1} P solutions. Phosphorus desorption into the solution occurred at these concentrations; this phenomenon was also described by Langmuir (20) under P saturated conditions. Thus, if exposure concentration was likely to be > 100 mg l^{-1}, fine calcined clay would be the more effective substrate for P sorption, because of its greater capacity for P sorption (S_{max} = 462.9 mg kg^{-1}). Brick substrate was not as effective as either of the CCs examined. It may still be useful as a root-bed media for P sorption in subsurface-flow CWS, but it would have to be refreshed more often than the CCs, because its lower S_{max} (6.79 mg kg^{-1}) indicated that it does not have the capacity to sorb as much P as the CCs.

When the K (1.62) and S_{max} (6.79 mg kg^{-1}) of brick were compared with the other media screened, it was found to have a much lower S_{max}. Drizo et al. (6) screened several substrates and found a range of S_{max} values from 420 mg kg^{-1} P for lightweight expanded clay aggregates to 860 mg kg^{-1} P for a coarse fly ash. Xu et al. (7) screened fly ash, from a different source, and found an S_{max} of 8,810 mg kg^{-1}, which is an order of magnitude greater than that of Drizo et al. (6). However, fly ash increased the pH of its solution to >12, which also increased formation of insoluble calcium-phosphate complexes that precipitated from the solution and increased the S_{max}. Özacar (13) examined calcined alunite's sorption capacity and found its S_{max} = 118 mg kg^{-1}. The sorption capacity of brick is well below those of the substrates discussed above. When the S_{max} of fine CC (462.9 mg kg^{-1}) and coarse CC (256.3 mg kg^{-1}) were compared other substrates; only fly ash had an S_{max} that exceeded fine CC, and the S_{max} of coarse CC was similar to many of the previously screened substrates.

Even though the sorption capacity of brick was much lower than that of many screened media, it could still provide enough P sorption capacity to adequately remove P from lower concentration runoff or wastewater. Brick could be used in situations where nutrient concentrations in effluent are between 0.5 and 50.0 mg l^{-1}, the range in which its removal efficiencies were the greatest. Since brick is relatively cheap ($50/ton), it may be a more economical substrate for CWS with lower inflow volumes and nutrient concentrations. The CC is very efficient at sorbing P, but costs around $300/ton. The coarse CC recovered a larger proportion of the P supplied when compared with fine CC at concentrations < 100 mg l^{-1}. This indicates that for many CWS, coarse calcined clay would provide adequate P sorption and greater handling ease.

Substrate P desorption

Desorption from each substrate was evaluated at individual exposure concentrations. Phosphorus concentrations desorbed did not change between 24 and 48 h ($P = 0.769$); thus all figures and tables present averages of 24 and 48 h data. Brick P desorption differed from fine and coarse CC at all exposure concentrations ($P < 0.0001$; Fig. 2.3). Coarse and fine CC desorbed more P than brick at all exposure concentrations except 1,000 mg l^{-1} P. This trait may indicate that P bound to brick is less likely to desorb and thus more strongly bound than the P bound to CC, or it could be indicative of the disparity of sorption at lower P exposures, where CCs sorbed considerably more P than brick.

The relative P_R capacities of the substrates were significantly different ($P < 0.0001$), with the fine CC retaining the highest concentration of sorbed P, followed by coarse CC, and lastly brick (Table 2.2). Since the P_R relates to the initial amount of P sorbed, the high P_R of fine CC correlated highly ($P < 0.0001$) with its capacity for sorbing more P than the other substrates. The f and the slope obtained from plotting P_{ad} vs. P_R and forcing the intercept through zero was a better comparison of the relative P_R capacities of each substrate. Analyses comparing f and the slope for each substrate indicated that fine and coarse CC retained similar percentages of P sorbed at 99.8 ± 0.006 %, while brick retained only 94.6 ± 0.01 % of its initially sorbed P.

The three substrates examined desorbed only low levels of P, with the lowest $f >$ 95.1%. When we compared our substrate P_R with those determined in other studies, even brick shows a greater propensity for retention than the best media examined by Pant et al. (12), whose work screening several media for their sorption capacity resulted in a range of P_R from 56% for Lockport dolomite to 92% for a Fonthill sand. This indicates that the media screened were less likely to desorb P when P concentrations in water were lower than those necessary for sorption to occur. However, most studies that examine P sorption to different media do not examine desorption parameters so it is difficult to draw further comparative conclusions from these desorption data.

Substrate equilibration period

Equilibration time necessary for maximal sorption of P by each substrate was evaluated with steady exposure concentrations. Calcined clays and brick were exposed to 500 and 250 mg l^{-1} P, respectively, for eight days. Exposure concentration was not an important factor when analyzing these results. Instead, the focus was on time necessary for greatest P sorption. Coarse CC sorbed P from solution maximally after 72h of exposure (Fig. 2.4), and fine CC sorbed the most P from solution after 48h. Brick sorbed equivalent concentrations of P from solution after only 24h of exposure. Even though both CC substrates sorbed larger quantities of P than brick during the sorption experiments, brick had the fastest equilibration time with P during this experiment. Thus, even though brick P sorption capacity is lower than CC, it sorbed P more quickly. These results indicate that brick may be more useful than CC when hydraulic retention time is short. The CC would be useful in CWS settings with longer retention times or in situations where higher P loading rates make incorporation of a substrate with higher P sorption capacity critical.

Conclusions

This study showed that CCs could be especially effective substrates for P sorption in the root-bed media of CWS because of their high P sorption and retention capacities. The Langmuir S_{max} for fine CC was the largest at 462.9 mg kg^{-1} P. The coarse CC S_{max} was only 256.3 mg kg^{-1} P. Even though coarse CC had a lower S_{max} in comparison with the fine CC, it sorbed approximately 76 ± 2.6% of P supplied at exposure concentrations between 0.5 and 100 mg l^{-1} P, while the fine CC sorbed 68 ± 2.8 % of P supplied at the same concentration range. Fine CC's higher S_{max} can be associated with its ability to sorb greater quantities of P under higher exposure concentrations. At these higher concentrations, the coarse CC was saturated and actually desorbed P into the exposure solutions. Both coarse and fine CCs desorbed < 2% of the P sorbed during the sorption experiments.

Brick had a very low Freundlich K, 1.62, indicating brick did not sorb as much P as did CCs. Further, brick was not able to sorb P as strongly as the CC at any P concentrations. It was able to remove only 19.7 ± 2.5% of the P supplied during sorption experiments at P concentrations < 100 mg l^{-1}. Brick desorbed < 5% of P sorbed during initial exposures. Even though brick does not have a large P sorption capacity, it is able to sorb P more quickly than CC. Indicating, it may be a useful substrate in CWS with lower influent P concentrations or shorter retention times. CCs have a large capacity for P sorption. Coarse CC sorbs a greater proportion of P at exposure concentrations < 100 mg

l^{-1}, so as long as P levels in runoff/wastewater do not exceed this concentration, coarse CC is the better choice for a P sorbing material. Not only is it easier to handle, but the larger particle size also decreases the probability that a large storm event will facilitate loss of substrate via particle suspension. These substrates should be further evaluated in long-term mesocosm or field-scale studies to characterize more fully their sorption capabilities over time and to evaluate the impact of P loading and flow rate on substrate life span.

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References

(1) Nosengo, N. Fertilized to death. *Nat.* 2003, *425*, 894-895.

(2) Owen, J.S. Jr., Warren, S.L., Bilderback, T.E., Albano, J.P. Industrial mineral aggregate amendment affects physical and chemical properties of pine bark substrates. *HortSci*. 2007, *42*, 1287-1294.

(3) Headley, T.R., Huett, D.O., Davison, L. The removal of nutrients from plant nursery irrigation runoff in subsurface horizontal-flow wetlands. *Wat. Sci. Tech.* 2001, *44*, 77-84.

(4) Taylor, M.D., White, S.A., Chandler, S.L., Klaine, S.J., Whitwell, T. Nutrient management of nursery runoff water using constructed wetland systems. *HortTech*. 2006, *16*, 610-614.

(5) Ahn, C., Mitsch W.J. Scaling considerations of mesocosm wetlands in simulating large created freshwater marshes. *Ecol. Eng.* 2002, 18, 327-342.

(6) Drizo, A., Frost, C.A., Grace, J., Smith, K.A. Physico-chemical screening of phosphate-removing substrates for use in constructed wetland systems. *Wat. Res.* 1999, *33*, 3595-3602.

(7) Xu, D., Xu, J., Wu, J., Muhammad, A. Studies on the phosphorus sorption capacity of substrates used in constructed wetland systems. *Chemos.* 2006, *63*, 344-352.

(8) Casey, R.E., Taylor, M.D., Klaine, S.J. Mechanisms of nutrient attenuation in a subsurface flow riparian wetland. *J. Env. Qual.* 2001, *30*, 1732-1737.

(9) Hinesly, R.L., Jones, T.D. Phosphorus in waters from sewage sludge amended lysimeters. *Env. Poll.* 1990, *65*, 293-309.

(10) US Environmental Protection Agency. *Quality criteria for water*. USEPA Rep. 440/5-86-001. USEPA Office of Water Regulations and Standards. U.S. Gov. Print. Office, Washington, DC. 1988.

(11) Kröger, R., Holland, M.M., Moore, M.T., Cooper, C.M. Plant senescence: A mechanism for nutrient release in temperate agricultural wetlands. *Env. Poll.* 2007, *146*, 114-119.

(12) Pant, H.K., Reddy, K.R., Lemon, E. Phosphorus retention capacity of root bed media of sub-surface flow constructed wetlands. *Ecol. Eng.* 2001, *17*, 345-355.

(13) Özacar, M. Adsorption of phosphate from aqueous solution onto alunite. *Chemos.* 2003, *51*, 321-327.

(14) Prochaska, C.A., Zouboulis, A.I. Removal of phosphates by pilot vertical-flow constructed wetlands using a mixture of sand and dolomite as a substrate. *Ecol. Eng.* 2006, *26*, 293-303.

(15) Vohla, C., Alas, R., Nurk, K., Baatz, S., Mander, Ü. Dynamics of phosphorus, nitrogen and carbon removal in a horizontal subsurface flow constructed wetland. *Sci. Tot. Env.* 2007, *380*, 66-74.

(16) Kim, J., Mann, J.D., Kwon, S. Enhanced adsorption and regeneration with lignocellulose-based phosphorus removal media using molecular coating nanotechnology. *J. Env. Sci. Health Pt. A.* 2006, *41*, 87-100.

(17) Bar-Yosef, Kafkafi, U., Rosenberg, R., Sposito, G. Phosphorus adsorption by kaolinite and montmorillonite: I. Effect of time, ionic strength, and pH. *Soil Soc. Am. J.* 1988, *52*, 1580-1585.

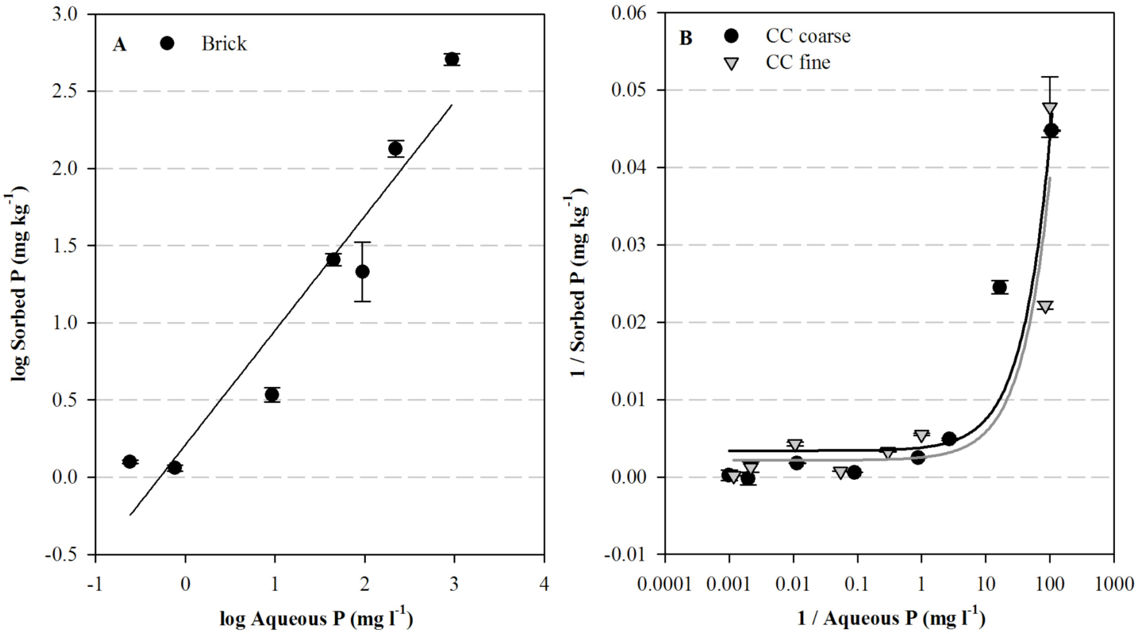
(18) Brady, N.C., Weil, R.R. *The Nature and Property of Soils.* 12th ed. Prentice Hall, London. 1990.

(19) Brick Industry Association. Manufacturing, classification, and selection of brick. Technical Notes 9. Reston, VA. 2004.

(20) Langmuir, D. *Aqueous Environmental Geochemistry.* Prentice Hall. Upper Saddle River, NJ. 1997.

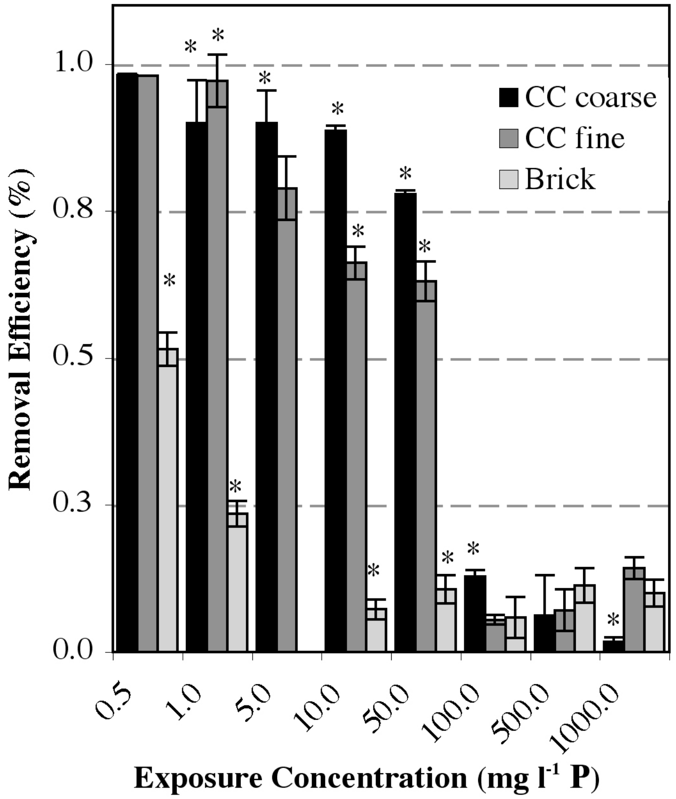
(21) Woodruff, J.R., Kamprath, E.J. Phosphorus adsorption maximum as measured by Langmuir isotherm and its relationship to phosphorus availability. *Soil Sc. Soc. Am. Proc.* 1965, 29, 148-150.

Figure 2.1. Relationship between equilibrium solution P and sorbed P for substrates[a] examined. (A) Depicts brick P sorption relationship with the Freundlich model, and (B) depicts the fine and coarse CC relationship with the Langmuir model.



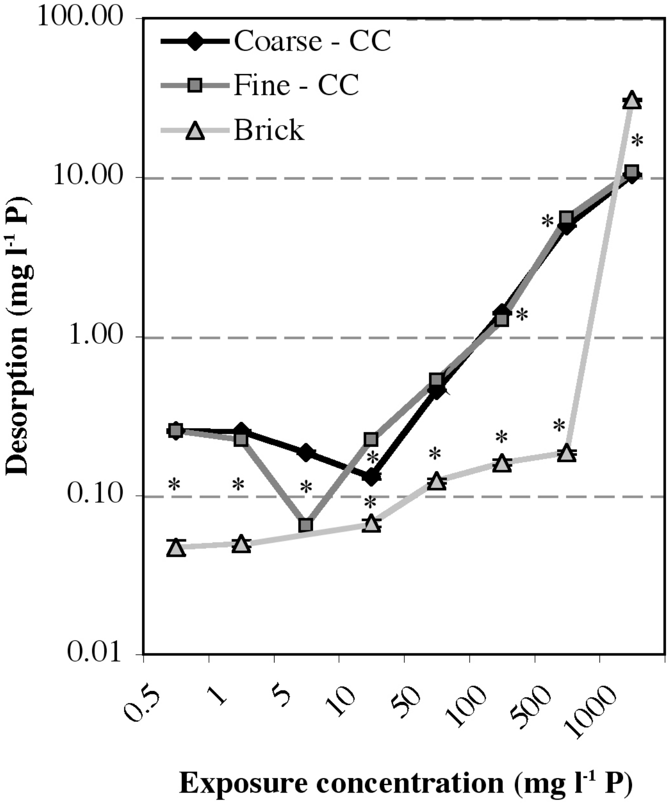
[a]CC = calcined clay. Values are the mean of 6 replicates ± the standard error of the mean per exposure concentration.

Figure 2.2. Substrate P removal efficiency (percentage P sorbed vs. P available) evaluated with various P exposure concentrations.[a]



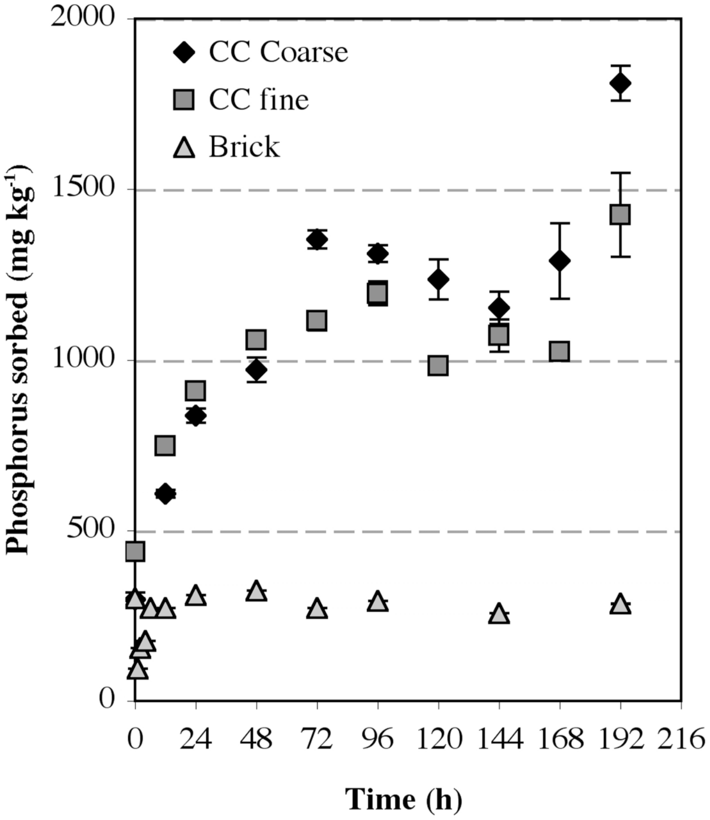
[a] Bars with * are statistically different at the specified exposure concentration ($P < 0.05$). CC = calcined clay. Values are the mean ± standard deviation of the mean of 6 replicates per exposure concentration.

Figure 2.3. Relationship between P sorbed to substrate and P desorption over average of 24 and 48 h equilibration period. [a]



[a] Statistically significant difference (*) at specified exposure concentration ($P < 0.05$). CC = calcined clay. Values are the mean ± standard deviation of the mean of 6 replicates per exposure concentration.

Figure 2.4. Relationship between time and substrate[a] sorption of P with fixed exposure concentrations.



[a] CC = calcined clay. Values are the mean ± standard error of the mean of 6 replicates per exposure concentration.

Table 2.1. Freundlich and Langmuir coefficients derived from sorption isotherms for substrates tested. [a]

	P_{ad}	Freundlich Parameters			Langmuir Parameters		
	(mg kg^{-1})	*b*	K	r^2	S_{max}	*k*	r^2
Brick	102.43 (71.56)	0.74	1.62	0.957	6.79	0.76	0.790
Coarse CC	497.09 (227.99)	0.31	180.64	0.843	256.28	9.79	0.934
Fine CC	1239.47 (737.84)	0.39	175.91	0.908	462.85	5.92	0.940

[a] CC = Calcined clay industrial aggregate. Only the positive portions of the slopes were used to characterize the sorption parameters, if saturation occurred, that negative slope was not included in the calculations. P_{ad}, average P adsorbed to substrate; *b*, slope; K, Freundlich sorption coefficient; S_{max}, sorption maxima; *k*, binding strength.

Table 2.2. Average of P retained by substrate after 24 or 48 h equilibration with ionic solution. [a]

Substrate	Slope	r^2	Mean P_r	*f*
Brick	0.947	0.9996	95.65 (44.31)	0.963 (0.009)
CC coarse	0.998	1.00	495.15 (177.31)	0.986 (0.004)
CC fine	0.998	1.00	1230.12 (500.62)	0.988 (0.002)

[a] CC, Calcined clay industrial aggregate; Mean P_R (mg P kg^{-1} substrate), $P_{adsorbed} - P_{desorbed}$ after 24 and 48 h equilibration; $P_R = P_{ad} * f$; slope, mean fraction of P_R; *f*, ratio of $\frac{P_R}{P_{ad}}$.

CHAPTER 3: HYDRAULIC RETENTION TIME, CONCENTRATION, AND INDUSTRIAL MINERAL AGGREGATE IMPROVE NUTRIENT REMEDIATION EFFICIENCY OF CONSTRUCTED WETLAND SYSTEMS

Abstract

Nutrient rich runoff from landscape nursery production areas is coming under increased scrutiny and is likely to be regulated in the near future. Constructed wetland systems (CWS) are management systems that can assimilate and remove nutrients from the runoff. This study evaluated how nutrient loading and hydraulic retention time impacted nutrient removal efficiencies in CWS. Simulated nursery runoff water with N concentrations < 20 mg/L N were efficiently treated in surface-flow CWS; if N concentrations were ≥ 28.5 mg/L adequate treatment did not occur. Instead net export of ≥ 10 mg/L N occurred from October through March. Secondary subsurface-flow mesocosms lined with industrial mineral aggregate were able to reduce P export by 60 – 74 % until the media saturated during April and May. Sequential extractions of the saturated media indicated that the majority of the P was associated with the Ca and Mg extractable fraction and that < 4.0 ± 4.0 % of P was freely exchangeable or available for plant uptake. The 4-day hydraulic retention time provided the most consistent nutrient removal efficiencies, and reduced P concentrations exported during winter months. This surface-flow to subsurface-flow CWS strategy shows great potential for reducing nutrient

exports from nursery runoff as long as average nutrient loading is accounted for when planning CWS size.

Introduction

Nutrient contamination from non-point sources is garnering increased attention in public, private, and governmental sectors because of the very noticeable downstream effects that even minor increases in nitrogen (N) and phosphorus (P) can produce. Non-point source contributors of nutrients include runoff from agricultural, forested, and urban areas. Even slight increases in nutrient concentration in water bodies can increase the rate of eutrophication. Effects of increased eutrophication can range from slightly increased primary productivity, both phytoplankton and aquatic macrophytes, to expanding dead zones in estuaries, bays, and seas that are a result of very low dissolved oxygen levels from excess nutrient loading brought to estuarine areas by rivers (1, 2).

Nutrient runoff from nursery and greenhouse production areas are of interest, because these industries are the largest per unit area users of fertilizers and pesticides (2). Nursery effluent can range in concentrations from 0.1 to 135 mg/L NO_3-N and 0.01 to 20 mg/L P (2-6). Constructed wetland systems (CWS) are low-cost treatment options that if implemented by nursery operators would reduce nutrient effluent concentrations. Many CWS evaluated recently utilize one species, such as cattail, common reed, or bullrush (6-8). This monospecific plant-stand strategy does not account for differential nutrient uptake capacities of multiple plant species. Mixed-plant CWS enhance ecosystem function and may retain up to 30% more nutrients (7, 9).

Nitrogen is efficiently assimilated in constructed wetlands and released as N gas via nitrification-denitrification processes (10). Phosphorus removal is more variable, and is aided to varying degrees by plant uptake, accretion into wetland soils, sorption to sediment, precipitation reactions, and immobilization by microbes (10-13). Sustained phosphorus removal in CWS is low unless substrates with high sorption capacities are used. To ultimately remove excess phosphorus from wetland cycling, both plant harvest and saturated sediment removal are needed. However, the utility of plant harvest is not consistently agreed upon; Kim and Geary (14) found that plant harvest did not dramatically improve phosphorus remediation efficiency (PRE) because the energy required for plant regrowth and the time taken for plants to regrow after harvest reduced the overall PRE. However, plant harvest is still considered by many researchers as a means to remove assimilated phosphorus because of the considerable quantities of P that reside in plant mass and can be exported during plant senescence (15-16).

Seo et al. (17) evaluated oyster shell additions to various filter substrates and saw P adsorption (P_{ad}) ranging from 180 to 7925 mg/kg based on oyster shell percent. Other media including gravel, dolomite, furnace slag, fly ash, shale, limestone, and sand have been incorporated into subsurface-flow CWS and have improved their P removal efficiencies from 10 to 71% (12, 17-20). However, increased PRE does not necessarily coincide with lower effluent P concentrations because the substrate equilibrium concentration (where no net sorption or desorption occurs) may be at concentrations higher than desired effluent levels (12). Akratos and Tsihrintzis (8) evaluated the effect of hydraulic retention time (HRT) on nutrient removal efficiency and found that 8-day

HRTs were adequate for P fixation as long as temperature was above 15° C. If temperature was below 15 °C 14- to 20-day HRTs were necessary.

Many factors drive nutrient cycling in CWS. Water temperature, inflow concentration, inlet flow rate, and biogeochemical nutrient cycling all contribute to nutrient cycling patterns (21). Objectives of this research were to characterize the influence of hydraulic retention time and influent nutrient concentration on N and P attenuation in surface-flow CWS and to determine the P sorption capacity of industrial mineral aggregate (calcined clay, CC) and its potential for reducing effluent P concentration when used as the root-bed substrate in subsurface-flow CWS.

Experimental Procedures

Primary Mesocosm Experiment

Primary mesocosm setup used twenty-four 380-L mesocosms planted with aquatic macrophytes. Approximately 15.2 cm of granite pea gravel lined the bottom of each mesocosm. Macrophytes were planted in gravel and water levels were raised slowly to maximum fill level. Mesocosms were planted in late July 2005 and allowed to establish for two months before sampling began. Sampling occurred from 10 October 2005 to 22 May 2006.

Plant Composition

Approximately 4.5 ± 0.4 kg of live plant material was planted in each primary mesocosm and plant species included in mesocosms were *Sagittaria graminea* (duck potato), *S. latifolia* (arrow-head), *Pontaderia cordata* (pickerelweed), *Hydrocotyle ranunculoides* (water pennywort), *H. umbellata* (marsh pennywort), *Panicum hemitomum* (maidencane), *Murdannia keisak* (marsh dewflower), and *Canna flaccida* (Florida canna).

Simulated Nursery and Greenhouse Runoff

Nutrients were supplied to mesocosms via pump from 1135.6 L holding tanks. Simulated nursery and greenhouse runoff was prepared using a 20-2-20 Nitrate Special, commercial-grade, water-soluble fertilizer (Southern Agricultural Insecticides Inc., Hendersonville, NC) and a 0-52-34 Phosphate Special, water-soluble fertilizer (Haifa Chemicals, Israel). Three nutrient levels were used with eight mesocosm replicates for each level. Initial (October 2005) exposure concentrations were 26.1 mg/L N (NO_3-N, NH_3-N, and urea N) and 9.5 mg/L P (P_2O_5 and P) for the low treatment, 72.9 mg/L N and 13.4 mg/L P for the medium, and 146.6 mg/L N and 20.9 mg/L P for the high treatment. Thereafter (November 2005 – May 2006), exposure concentrations were reduced to 8.6 mg/L N and 2.9 mg/L P for the low, 18.0 mg/L N and 3.6 mg/L P for the medium, and 28.5 mg/L N and 4.2 mg/L P for the high to mimic more closely nutrient concentrations in nursery production runoff.

Hydraulic Retention Time

Flow rate into each mesocosm was adjusted to the desired flow rate for either a 4- or 7-day hydraulic retention time (HRT) whenever holding tanks were refilled. Flow rate into the 4-day HRT mesocosms averaged 70.9 L/day and flow rate into the 7-day HRT mesocosms averaged 40.6 L/day.

Secondary Mesocosm Experiment

Subsurface-flow, secondary mesocosm treatments were established by piping the discharge from eight primary treatment mesocosms into eight 190 L holding tanks filled with approximately 90.7 kg of a coarse (0.8 to 4.75 mm) industrial mineral aggregate (calcined clay). The calcined clay (CC) was mined in Ochlocknee, GA (Oil-dri Corp. of America, Chicago), and previous work with the substrate had identified it as a media with good P sorbing properties (Chapter 2).

Experimental Design

The design was a 2 x 3 factorial with six concentration by retention time combinations. Four replicates were used for each treatment in the primary mesocosm experiment. Primary mesocosm treatments were 1) 4-day High (H4), 2) 4-day Medium (M4), 3) 4-day Low (L4), 4) 7-day High (H7), 5) 7-day Medium (M7), and 6) 7-day Low (L7). High, medium, and low refer to the nutrient inflow concentration for each treatment. The secondary mesocosm experiment had two treatment combinations, a 4-day High (H42) and 7-day High (H72), with four replicates per treatment.

Sampling Endpoints

Water samples were taken weekly and analyzed for NH_3^+, NO_3^-, NO_2^-, PO_4, pH, non-purgable organic carbon (NPOC), total dissolved nitrogen (TN), water temperature (°C), conductivity (mVolts), and dissolved oxygen (DO). Anions were determined using a Dionex AS10 ion chromatograph with AS50 auto-sampler (Dionex Corp., Sunnyvale, CA). NPOC and TN were analyzed using a Shimadzu TOC-V $_{CPH}$ total organic carbon analyzer with TNM-1 total nitrogen measuring unit (Shimadzu Scientific Instruments, Kyoto, Japan), and ammonia was measured using Orion Ammonia Electrode 95-12 (Thermo Electron Corp., Beverly, MA).

Plant Harvest

At termination of the experiment, plant material was harvested from each mesocosm, water allowed to drain, and the wet weight of plant material removed from each mesocosm determined. Water displacement by plant material was calculated by dividing the volume of water remaining in mesocosms after plant material harvest by the volume of water required to refill mesocosms to outflow pipe.

Sequential P Extractions

Calcined clay samples were taken from three depths in the front and back of each secondary treatment mesocosm. The sampling depths were 1) the top 3 cm of CC after the bio-layer was removed, 2) the middle CC layer ~ 9 cm below first sample, and 3) the bottom CC layer ~ 9 cm below the middle layer. A series of sequential extractions was performed on each of the CC samples to determine plant available phosphorus, the relative proportion of P bound to each soil fraction, and total P sorbed to the CC (22).

Statistical Analyses

The data were analyzed using SAS PROC GLM with LSD means separation in October 2007 (SAS Institute Inc. Cary, NC). Correlation analyses were used to characterize relationships between dependent (N and SRP) and independent (temperature, conductivity, DO, pH, sulfate concentration, and NPOC) study variables. Correlation analyses were conducted using SAS PROC CORR to evaluate parameter correlation and detect multicolinearity if present among parameters. Those variables that correlated with each other were input into stepwise regression models using SAS PROC REG.

Results and discussion

Removal efficiency of N in primary mesocosms

Nitrogen dynamics in the primary mesocosms were monitored. The results displayed were averaged by month (with four sampling periods per month). For greater detail, individual sampling results for N and P are shown in the supplemental figures (Fig. S3.1 - S3.3). Low, medium, and high treatments showed increasing total N (NO_2 + NO_3 + NH_3 mg/L) removal efficiency after January 2006 (Fig. 3.1). Decreased N removal efficiencies for high treatments in October may be attributed to a number of factors including high nitrogen loading rates, insufficient establishment time for macrophytes, and insufficient acclimation time for the microbial colonies responsible for denitrification (Table 3.1). During October, the high treatment inflow concentration was 146.6 ± 10.9 mg/L N, and total October N loading into H4 and H7 mesocosms (1,038.1 ± 41.4 and 714.9 ± 31.3 g N respectively) was comparable to total N loaded into H4 and H7 for the November to May sampling period (1,369.7 ± 17.4 and 822.1 ± 11.2 g N respectively; Table 3.1). During November, high treatment nitrogen removal efficiency (NRE) was positive; this was also when inflow N concentrations were decreased to more closely mimic runoff from nursery production areas.

Low treatment removal efficiencies were all positive (Fig. 3.1) except during December when the L4 treatment exported N. Not only were N inflow concentrations not reduced, it is likely that plant senescence contributed N; thus, excess N was exported.

During December water temperatures were ~ 6.2 ºC (Fig S3.4). As water temperature increased with the arrival of spring in late March, N removal efficiency for all treatments increased. These findings agree with other researchers who have documented seasonal trends in field scale wetlands, where N removal efficiencies decrease as water temperature falls below 15 ºC (2, 13, 23).

Removal efficiencies were similar among treatments by February and March except for H4 and H7, which still had consistently lower N removal efficiencies, and N outflow concentrations of 21.7 ± 1.1mg/L total N in February and 16.5 ± 1.6 mg/L total N in Mar (Fig. 3.1). These outflow concentrations were well above the water quality standard of 10 mg/L NO_3 and 1 mg/L NO_2 (24), which could indicate that mesocosm treatment area was insufficient to handle that loading rate during winter months. M4 and M7 outflow N concentrations were 11.1 ± 1.2 and 10.3 ± 1.2 mg/L total N during February; thereafter M4 and M7 outflow concentrations were ≤ 10 mg/L total N. L4 and L7 outflow concentrations were well below 5.0 mg/L total N for January, and from February through April L4 and L7 outflow concentrations were ≤ 2.7 ± 0.2 and 2.1 ± 0.2 mg/L total N, respectively. By May all treatments had similar NRE and N export concentrations were ≤ 0.6 mg/L total N, except H4 which had total N export of 2.0 ± 0.8 mg/L.

Influent nutrient concentration was a factor in mesocosm total N removal efficiency, especially during winter months. High treatment mesocosms exported N at concentrations much higher than those shown to contribute to increased eutrophication in surface waters. The medium and low treatment mesocosms were able to efficiently reduce total N exports at this experimental scale. From December through March high treatment NRE was too low to reduce N export to acceptable levels.

Hydraulic retention time was only a major factor during winter months (December through February) when 7-day HRT showed consistently more efficient N removal than 4-day HRT. The higher removal efficiencies of L7, M7, and H7 during December and January can be attributed to the longer retention time, which may have enabled microbial colonies to denitrify more of the loaded N (Table 3.2, Fig. 3.1). Akratos and Tsihrintzis (8) found similar results with HRT >14 days necessary for sufficient NRE efficiencies with lower temperatures. During the fall of 2005 and spring of 2006, NRE of the 4- and 7-day HRT were similar among treatments. It appeared that the rates of plant N uptake and microbially facilitated denitrification in the 4-day HRT were fast enough to have equivalent NREs and N export concentrations when compared with the 7-day HRT treatment systems. These results differed from those of Akratos and Tsihrintzis (8) who found that NRE in subsurface-flow mesocosms with 6-day HRTs was significantly lower than those with 8-day HRTs. However, although some statistical differences existed between NREs for 4-and 7-day HRT by inflow concentration, the differences were not large enough to suggest that a 7-day HRT should be used instead of a 4-day HRT. The surface area required for a CWS with a 7-day HRT would be much larger than that required for a 4-day HRT. Thus, if a nursery or greenhouse operation were to install a CWS to treat their runoff, the NRE difference between 4- and 7-day HRT would not justify taking the extra land from production to build a 7-day HRT CWS.

Removal efficiency of N in secondary mesocosms

Evaluating P removal was the objective for using secondary treatment mesocosms, but to ensure that secondary treatment did not increase N export from mesocosms, N concentrations were also monitored. Treatment mesocosms H42 and H72 exported N from November through December (Fig. 3.2). Thereafter, treatments were similar with no effect of HRT on NRE. However, total N remediation efficiency increased significantly in H42 and H72 treatments from February to March, but thereafter, no consistent pattern emerged. It appeared that export from secondary treatments occurred when primary treatments cells were exporting (Figs 3.1 and 3.2). Secondary treatment resulted in no net increase or decrease in total N removal efficiency.

Remediation efficiency of SRP in primary mesocosms

Soluble reactive phosphorus removal in CWS was not controlled by the same factors that had heavily influenced N remediation. Microbial transformation is not a primary removal mechanism for P. Sorption of P onto substrates and sediments is the primary removal mechanism for P, followed by plant uptake (usually < 5% of P removal), accretion into wetland soils, and precipitation reactions (11-12). Temperature did not influence P removal efficiency (PRE); instead, the only seasonality effect noticed was when active plant growth facilitated greater PRE.

Positive SRP removal efficiency occurred in October for all primary treatments (Fig. 3.3) with no differences in removal efficiency between 4- and 7-day HRT (Table 3.3). This positive October SRP removal efficiency was remarkable, considering P loading into the mesocosm during October was similar to the combined P loading for the period December to May (Table 3.1). This positive removal efficiency may be attributed to active plant growth, since the macrophytes were still actively growing and filling in the mesocosms. The two-month establishment period may not have been long enough for plants in mesocosms to attain their full size, so additional P may have been removed as a result. Thereafter, until April all treatments exported some SRP. Average P export from November to March was 4.2 ± 0.1 mg/L for L4 and L7, 5.3 ± 0.1 mg/L for M4 and M7, and 7.3 ± 0.2 mg/L for H4 and H7. Four-day HRTs resulted in less SRP export than 7-day HRTs. This may be an artifact of dilution as the faster flow rate in 4-day HRTs dilutes P released from decaying plant material. In April, SRP removal became positive in L7, M4, M7, and H4 but SRP was still being exported from L4 and H7. In May all were positive except M4 and H4. However, since P concentrations were highly variable in both M4 and H4 treatments (magnitude of standard error bars), positive SRP removal may have occurred, even though the average removal efficiency did not reflect this trend.

Primary treatment alone was not able to provide adequate SRP removal; for all treatments, five of the eight months sampled showed significant export. Not only was SRP from inflow not being fixed, internal cycling processes were contributing SRP, resulting in a net export of SRP. Other researchers have reported similar findings, in that SRP is not consistently managed via CWS because internal phosphorus cycling often leads to SRP export (2, 4, 15). Therefore, a secondary treatment system is necessary to better manage SRP removal.

Remediation efficiency of SRP in secondary mesocosms

Secondary, subsurface flow mesocosms with a CC root-bed substrate were monitored to assess substrate P sorbing capacity and longevity. The assessment was divided into two areas: 1) system (SYS), which represented SRP removal efficiency from inflow load to secondary treatment outflow, and 2) secondary (SEC), which represented SRP removal efficiency from primary treatment outflow to secondary treatment outflow. Sorption of SRP by CC in October was highly efficient; however, since loading was extremely high (Table 3.1), many P sorption sites were saturated during the first exposure month. Both SEC and SYS P removal efficiencies were ~ 60 % with average system outflow concentrations of 7.3 ± 0. 6 mg/L P (down from 17.8 ± 1.1 mg/L P primary outflow, Fig. 3.4). During October, for each 90.7 kg of CC substrate in secondary mesocosms, approximately 81.5 ± 2.2 g of P were fixed in H42 treatments and 65.8 ± 1.7 g of P were fixed in H72 treatments.

Calcined clay sorption capacity was characterized in previous work (Chapter 2), and the maximum amount of P was sorbed (1740.5 ± 6.6 mg P / kg CC) by the CC when aqueous P concentrations were 50 mg/L. This maximum P_{ad} indicated saturation of the secondary treatments filled with 90.7 kg of CC should occur when 157.9 g of P had been bound. The results of this study did not agree with this prediction. However, the predicted binding did not take into account migration of P from external binding sites to internal sites over time, which would leave previously saturated sites available for further P sorption. The possibility also exists that the CC lot used in this study may have a higher SRP sorption capacity than the previously screened lot. If the latter possibility was the main factor controlling P binding, pre-screening CC lots for their P sorption ability may be necessary when using it as a secondary subsurface-flow root bed substrate.

Because 98.5 ± 1.9 g of P were fixed in October by both H42 and H72, a rapid decline in PRE was expected. However, PREs from November through March were still greater than primary treatments, resulting in lower P outflow concentrations (Fig. 3.4). From November through March SRP removal was efficient with SEC for H42 and H72. After March SEC PRE was no longer positive and the substrate began to desorb SRP. In April and May, SRP desorption occurred for both H42 (-45.5 ± 4.1 g P) and H72 (-39.9 ± 2.0 g P), resulting in a SYS P outflow much greater than P loaded from the primary treatment. In May, both SEC and SYS for H42 were highly negative (~ -2700%) and 6.1 ± 1.6 mg/L P was exported from secondary treatments, while primary outflow P concentrations were 0.75 ± 0.94 mg/L. The H72 P export concentration in May was 8.0 ± 0.2 mg/L P, while primary treatment outflow concentration was only 0.2 ± 0.01 mg/L P. At this point (early April) both H42 and H72 were saturated and rapid desorption occurred because primary treatment outflow P concentrations were low because of increased plant P uptake during active growth. These findings agreed with those of Pant et al. (12) and indicated that even though the CC had a large sorption capacity, its equilibrium P concentration, where no net sorption or desorption occurred, was higher than the desired P export concentration. The increase in P export from H42 and H72 in April and May was expected. However, since the H72 treatment had not fixed as much SRP as H42 in October and from November to March, it was anticipated that the CC substrate would be able to sorb additional SRP. Instead, SRP desorption from the H72 treatment occurred concurrently with SRP desorption from the H42 treatment, and the mass desorbed was only 5.6 g less than that desorbed from H42 during April and May (Fig. 3.4, Table 3.1, and Table 3.4). This happened because of the initially high P loading rate into H42 and H72 that caused an increase in the P equilibrium concentration of CC, where no net sorption or desorption occur. This increased P equilibrium concentration lead to higher P effluent concentrations. Pant et al. (12) found that equilibrium P concentrations of shale and sand substrates increased over time, resulting in higher effluent P concentrations.

Total SRP fixation at the conclusion of the experiment was 98.5 ± 4.9 g P for H42 and 71.4 ± 3.8 g P for H72; masses very similar to the SRP fixation that occurred in October. Secondary treatment mesocosms were very effective at removing SRP from effluent during October and November with a 2.9 fold increase in removal efficiency over the primary treatments. The majority of the SRP that could be sorbed was fixed in the first month, making long-term predictions about SRP sorption and PRE difficult. An experiment with consistent P loading rates should be conducted to predict with greater accuracy the lifetime of the substrate based on SRP loading rate and weight of CC used as root bed substrate. This current experiment showed an increase in P removal efficiency with the use of CC as a substrate, but SRP concentrations in secondary treatment effluent were still much higher than the recommended 0.05 mg/L PO_4-P and 0.1 mg/L organic P for effluent (24-25). Further experiments examining secondary treatment size based on SRP loading rate should be performed so that consistent remediation can be achieved when applying this technology to larger scale CWS.

Water displacement and primary treatment affects on plant mass

Upon termination of the experiment, plants were harvested and fresh weights were used to determine if nutrient treatment impacted plant mass in mesocosms. When the treatment averages (Fig. 3.5) were evaluated; it was found that there were slight differences, but they could not be attributed to treatment effects alone. The mesocosm treatment with the largest plant mass at harvest was L4, while H4 had the smallest plant mass at harvest. Plant mass at harvest in the mesocosms was not attributable to nutrient inflow concentration or HRT.

Biomass displacement in the mesocosms was calculated. After biomass removal, excess water drained from roots removed from each mesocosm was returned to the mesocosm. The number of liters remaining in a mesocosm was subtracted from the number of liters required to fill the mesocosms to its outflow pipe and this number was used as a measure of percent displacement by biomass (Fig. 3.6). Nutrient loading rate treatment (high, medium and low) and HRT did not impact displacement. This information will facilitate CWS design by ensuring correct CWS size based not only on nutrient loading rate and desired HRT, but the added area needed to account for wetland biomass.

Sequential phosphorus extractions

Once P binding sites on CC substrate in secondary treatment mesocosms were saturated, samples were taken at three depths and sequential P extractions performed. The initial extraction utilized distilled water and an anion exchange membrane to quantify freely exchangeable phosphorus. No chemical modification of the substrate solution was involved (22). Very little P was extracted from CC during this step (Table 3.5). The second extraction utilized a 0.5 M $NaHCO_3$ solution, which removed weakly bound, plant-available P (22, 26). This P fraction was slightly larger than the previous (Fig S3.5) and > 2-fold more P was extracted from inflow locations than from outflow locations (Table 3.5).

The third extraction involved a 0.1 M NaOH solution and P obtained during this extraction is considered to be associated with Fe and Al (22, 26). The majority of the P extracted in this step was associated with Al (Table 3.6) because Al concentrations > 20% higher than Fe for both H42 and H72 treatments. The fourth extraction utilized 1 M HCl and extracted P associated with Ca and Mg (22, 26). This comprised the largest fraction of P extracted from CC (Table 3.5 and Fig. S3.5). Calcium concentrations in the CC (6,094 ± 172 mg/kg) were slightly higher than Mg (4,189 ± 66 mg/kg), and thus P may have associated slightly more with Ca than Mg. Since the majority of the P extracted from CC came from this step, it is likely that insoluble Ca compounds like hydroxyapatite formed and were a major factor controlling P fixation by CC. Since the pH was generally > 7.0 conditions in these secondary treatments would have favored Ca-P product formation rather than formation of Fe- and Al- compounds (26).

The last extraction step involved concentrated HCl and removed highly recalcitrant and residual P. The P concentration extracted during this step was much lower than the concentration extracted during steps 3 and 4 (Table 3.5). This reaffirmed that the majority of P extracted was associated with Ca and Mg. Thus, as long as effluent pH is basic, Ca and Mg will be the primary factors controlling P binding with this substrate, while if pH were to decrease; Al and Fe may become more important for P fixation.

Nutrient effluent concentration modeling

After determining multicollinearity was not extensive with total nitrogen (mg/L), phosphorus (mg/L), sulfate (mg/L), pH, temperature (°C), conductivity (mVolts), NPOC (mg/L), and nitrogen loading (g/day), stepwise regressions were performed, and those factors that significantly contributed to explaining model variability were fit with a regression model. Estimates of slope for each variable were then standardized, using the stb option of SAS PROC REG, so that the relative importance of each variable for estimating N effluent concentration could be determined. Around 89.3% of nitrogen effluent concentration variability was explained using these independent factors:

$$\begin{aligned}\text{Nitrogen effluent} = {} & 81.818 - 0.028{*}\text{sulfate} - 0.292{*}\text{pH} - 0.126{*}\text{temperature} + \\ & 0.912{*}\text{conductivity} - 0.114{*}\text{NPOC} - 0.039{*}\text{nitrogen load} + \\ & 0.107{*}\text{sulfate\_nitrogen load} + 0.177{*}\text{NPOC\_nitrogen load} \qquad [1]\end{aligned}$$

Detailed residual plots of each factor (except nitrogen load) by nitrogen effluent concentration adjusted for the other model variables can be found in the supplementary material (Fig. S3.6). Previous work by the authors examining nitrogen removal efficiencies in a large-scale CWS in Cairo, GA over a three-year period found that sulfate concentration correlated with NRE, but NPOC and water temperature, though highly correlated, did not consistently account for NRE variability. Our findings that temperature and NPOC were useful may be an artifact of the shorter sampling period. While model variability was explained by these independent factors, none of them could be easily manipulated to improve either NRE or PRE. Consequently, while useful for predicting nitrogen concentrations, none, save nitrogen load, could be used to decrease N effluent concentrations.

These independent parameters explained 87.1% of total P effluent concentration:

$$\begin{aligned}\text{Phosphorus effluent} = {} & 302.501 + 0.070{*}\text{sulfate} - 0.320{*}\text{date} + 0.077{*}\text{pH} - \\ & 0.170{*}\text{DO} + 0.583{*}\text{conductivity} + 0.057{*}\text{NPOC} + 0.039{*}\text{phosphorus load} \\ & + 0.097{*}\text{DO\_phosphorus load} + 0.035{*}\text{NPOC\_phosphorus load} \qquad [2]\end{aligned}$$

Detailed residual plots of each factor (except phosphorus load) by phosphorus effluent concentration adjusted for the other model variables can be found in the supplementary material (Fig. S3.7). Previous work by the authors exploring PRE examined similar independent factors and found that none could provide consistent explanation of PRE. Thus, even though the above variables were significant in the model, there was no real-world applicability for controlling/influencing PRE. Only NPOC could be altered, and its concentration was similar across the loading range for N and P. Thus, even though these models explained much of the NRE and PRE in this experiment, they cannot provide applicability for future N and P reduction.

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References

(1) Joyce, S. The dead zones: Oxygen-starved coastal waters. *Environ. Health Persp.*, 2000, *108*, A120-A125.

(2) Taylor, M.D.; White, S.A.; Chandler, S.L.; Klaine, S.J.; Whitwell, T. Nutrient management of nursery runoff water using constructed wetland systems. *HortTech.* 2006, *16*, 610-614.

(3) Yeager, T., Wright, R., Fare, D., Gilliam, C., Johnson, J., Bilderback, T., Zondag. R. Six state survey of container nitrate nitrogen runoff. *J. Environ. Hort.* 1993, *11*, 206-208.

(4) Headley, T.R., Huett, D.O., Davison, L. The removal of nutrients from plant nursery irrigation runoff in subsurface horizontal-flow wetlands. *Water Sci. Tech.* 2001, *44*, 77-84.

(5) Alexander, S.V. Pollution control and prevention at containerized nursery operations. *Wat. Sci. Tech.* 1993, *28*, 509-517.

(6) Huett, D.O., Morris, S.G., Smith, G., Hunt, N. Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands. *Water Res.* 2005, *39*, 3259-3272.

(7) Kao, J.T., Titus, J.E., Zhu, W.X. Differential nitrogen and phosphorus retention by five wetland plant species. *Wetlands.* 2003, *23*(4), 979-987.

(8) Akratos, C.S., Tsihrintzis, V.A. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. *Ecol. Eng.* 2007, 29, 173-191.

(9) Engelhardt, K.A.M., Ritchie, M.E. Effects of macrophyte species richness on wetland ecosystem functioning and services. *Nature.* 2001, *411*, 687-689.

(10) Vymazal, J. Removal of nutrients in various types of constructed wetlands. *Sci. Tot. Env.* 2007, *380*(1-3), 48-65.

(11) Richardson, C.J. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science.* 1985, *228*(4706), 1424-1427.

(12) Pant, H.K., Reddy, K.R., Lemon E. Phosphorus retention capacity of root bed media of sub-surface flow constructed wetlands. *Ecol. Eng.* 2001, *17*, 345-355.

(13) Kadlec, R., Knight, R. *Treatment Wetlands.* CRC Press. 1996. Boca Raton, FL.

(14) Kim, S.Y., Geary P.M. The impact of biomass harvesting on phosphorus uptake by wetland plants. *Wat. Sci. Tech.* 2001, *144*(11-12), 61-67.

(15) Ahn, C., Mitsch, W.J. Scaling considerations of mesocosm wetlands in simulating large created freshwater marshes. *Ecol. Eng.* 2002, *18*(3), 327-342.

(16) Kröger, R., Holland, M.M., Moore, M.T., Cooper, C.M. Plant senescence: A mechanism for nutrient release in temperate agricultural wetlands. *Env. Poll.* 2007, 146, 114-119.

(17) Seo, D.C., Cho, J.S., Lee, H.J., Heo, J.S. Phosphorus retention capacity of filter media for estimating the longevity of constructed wetland. *Wat. Res.* 2005, *39*, 2445-2457.

(18) Prochaska, C.A., Zouboulis, A.I. Removal of phosphates by pilot vertical-flow constructed wetlands using a mixture of sand and dolomite as a substrate. *Ecol. Eng.* 2006, *26*, 293-303.

(19) Vohla, C., Alas, R., Nurk, K., Baatz, S., Mander, Ü. Dynamics of phosphorus, nitrogen and carbon removal in a horizontal subsurface flow constructed wetland. *Sci. Tot. En.* 2007, *380*, 66-74.

(20) Xu, D., Xu, J., Wu, J., Muhammad, A. Studies on the phosphorus sorption capacity of substrates used in constructed wetland systems. *Chemos.* 2006, *63*, 344-352.

(21) Kadlec, R.H., Reddy, K.R. Temperature effects in treatment wetlands. *Wat. Env. Res.* 2001, *73*(5), 543-557.

(22) Tiessen, H.; Moir, J.O. Characterization of available P by sequential extraction. In *Soil sampling and methods of analysis.* Carter, M.R. Ed.; CRC press: Boca Raton. 1993, pp 75-86.

(23) Dahab, M.F., Surampalli, R.Y. Subsurface-flow constructed wetlands treatment in the plains: Five years of experience. *Wat. Sci. Technol.* 2001, *44*, 375–380.

(24) U.S. Environmental Protection Agency. Definitions: Water quality standards criteria summaries: A compilation of state/federal criteria. U.S. EPA. Office of Water Regulations and Standards. U.S. EPA-440/5-88/017. 1988, Washington, D.C.

(25) Hinesly, R.L., Jones, T.D. Phosphorus in waters from sewage sludge amended lysimeters. *Environ. Poll.* 1990, *65*,293-309.

(26) Headely, T.R., Huett, D.O., Davison, L. Seasonal variation in phosphorus removal processes within reed beds – mass balance investigations. *Wat. Sci. Technol.* 2003, *48*(4), 59-66.

Tables

TABLE 3.1. Total nitrogen (TN) and total phosphorus (TP) mean ± standard error removal / fixation[a] in surface- to subsurface-flow mesocosms. The experiment was broken into three sampling period groupings, October 2005, November 2005 to March 2006, and April to May 2006, based on loading rate.

OCT 2005	TN In	TP In	TN out	TP out	TN fixed system	TP fixed system	TN fixed secondary	TP fixed secondary
				(g)				
L4	210.83 (9.63)	70.02 (0.48)	434.55 (49.82)	57.10 (1.70)	-299.06 (51.57)	-33.11 (1.77)		
L7	112.73 (4.76)	44.71 (1.51)	267.56 (30.88)	30.01 (1.04)	-140.47 (27.03)	-9.66 (1.37)		
M4	590.92 (21.04)	106.64 (2.66)	714.04 (44.94)	82.64 (2.01)	-584.65 (40.99)	-58.31 (1.94)		
M7	312.37 (10.89)	58.20 (1.32)	351.07 (26.59)	52.43 (1.10)	-272.06 (25.87)	-38.71 (1.03)		
H4	1038.06 (41.43)	156.68 (4.66)	1383.54 (58.82)	138.48 (3.32)	-903.45 (81.28)	-47.29 (6.13)		
H7	714.93 (31.29)	97.35 (3.17)	839.93 (33.75)	89.92 (2.26)	-656.25 (46.77)	-58.83 (3.75)		
H42			1367.81 (57.56)	57.10 (2.00)	-887.72 (80.59)	-34.81(6.10)	15.73 (4.21)	81.47 (2.21)
H72			784.71 (36.48)	24.18 (1.47)	-601.02 (50.30)	6.92 (3.53)	55.23 (5.58)	65.75 (1.66)

NOV 2005 - MAR 2006	TN In	TP In	TN out	TP out	TN fixed system	TP fixed system	TN fixed secondary	TP fixed secondary
				(g)				
L4	337.66 (4.25)	98.28 (1.25)	622.26 (137.71)	149.18(2.54)	-123.23 (154.69)	-64.05 (5.73)		
L7	158.32 (1.30)	47.57 (0.42)	305.74 (80.04)	84.00 (2.10)	145.83 (123.30)	-1.07 (5.27)		
M4	666.74 (9.12)	122.07 (2.29)	834.16 (115.98)	177.76 (4.22)	-379.88 (124.65)	-86.79 (5.73)		
M7	332.82 (4.70)	61.50 (0.84)	386.08 (61.94)	103.95 (1.84)	93.75 (110.58)	14.91 (13.30)		
H4	966.87 (14.99)	134.80 (1.65)	1128.58 (76.50)	219.25 (7.91)	705.60 (169.75)	135.86 (38.22)		
H7	573.65 (10.20)	79.11 (0.98)	563.73 (56.11)	150.04 (3.79)	-40.23 (103.58)	-55.22 (9.29)		
H42			827.59 (33.61)	156.66 (3.85)	1006.60 (192.99)	198.45 (31.74)	301.00 (52.03)	62.59 (8.29)
H72			441.85 (24.78)	104.45 (5.40)	81.65 (44.22)	-9.63 (5.27)	121.88 (63.98)	45.58 (7.91)

APR-MAY 2006	TN In	TP In	TN out	TP out	TN fixed system	TP fixed system	TN fixed secondary	TP fixed secondary
				(g)				
L4	127.53 (3.78)	51.55 (1.06)	10.95 (1.37)	27.20 (1.42)	205.69 (15.08)	12.51 (2.81)		
L7	68.15 (2.91)	28.93 (1.84)	1.39 (0.29)	9.02 (0.61)	245.62 (18.29)	30.781 (1.61)		
M4	283.26 (11.26)	60.02 (3.04)	36.84 (3.34)	20.42 (1.60)	177.53 (16.98)	20.87 (2.43)		
M7	142.10 (5.59)	32.15 (1.53)	13.62 (8.67)	15.31 (0.81)	256.79 (15.72)	54.40 (2.94)		
H4	402.78 (19.85)	70.78 (3.62)	85.00 (8.67)	40.75 (2.76)	1231.37 (132.68)	167.23 (14.60)		
H7	248.40 (12.29)	39.89 (2.10)	46.71 (2.60)	24.77 (1.24)	162.58 (18.47)	17.75 (2.55)		
H42			125.82 (4.12)	86.27 (1.57)	1190.55 (128.75)	121.72 (12.55)	-40.82 (5.36)	-45.52 (4.10)
H72			52.96 (1.02)	64.70 (0.79)	156.33 (16.66)	-22.17 (2.02)	-6.25 (3.28)	-39.92 (1.96)

[a] System fixation calculated from $(T_{x, out} - T_{x, in})$; secondary fixation calculated from corresponding mesocosm ($H4(7)_{out} - H42(72)_{out}$). H4, high treatment 4 day hydraulic retention time (HRT); H7, high treatment 7 day HRT; M4, medium treatment 4 day HRT; M7, medium treatment 7day HRT; L4, low treatment 4 day HRT; L7, low treatment 7 day HRT; H42, high with secondary treatment 4 day HRT; H72, high with secondary treatment 7 day HRT. Low, medium, and high represent influent concentrations treatments.

TABLE 3.2. Total nitrogen removal efficiency statistics[a] for primary, surface-flow mesocosms from October 2005 to May 2006.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
L4	A	AB	BC	AB	A	B	AB	A
L7	A	A	A	A	AB	A	A	A
M4	B	B	B	B	BC	BC	BC	A
M7	B	AB	B	C	BC	B	AB	A
H4	C	AB	C	D	C	CD	C	B
H7	C	AB	B	C	C	D	C	A

[a] Statistical differences signified by different letters in the month columns; L4, low with 4 day HRT; L7, low with 7 day HRT; M4, medium with 4 day HRT; M7, medium with 7 day HRT; H4, high with 4 day HRT; H7, high with day HRT. Low, medium, and high represent influent concentrations treatments.

TABLE 3.3. Total phosphorus removal efficiency statistics[a] for primary, surface-flow mesocosms from October 2005 to May 2006.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
L4	AB	A	A	A	A	B	B	A
L7	A	BC	AB	DE	AB	AB	A	A
M4	AB	AB	AB	AB	A	A	A	B
M7	B	BC	B	BC	B	AB	A	A
H4	B	CD	B	CD	A	A	A	B
H7	B	D	B	E	AB	B	B	A

[a] Statistical differences signified by different letters in the month columns; L4, low with 4 day HRT; L7, low with 7 day HRT; M4, medium with 4 day HRT; M7, medium with 7 day HRT; H4, high with 4 day HRT; H7, high with day HRT. Low, medium, and high represent influent concentrations treatments.

TABLE 3.4. Total phosphorus removal efficiency statistics[a] for primary, surface-flow to secondary, calcined clay filled subsurface-flow mesocosms from October 2005 to May 2006.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
H4	A	A	AB	A	A	B	A	A
H7	A	B	AB	B	A	A	AB	A
H42 system	B	C	BC	C	A	B	AB	A
H72 system	BC	D	A	C	A	C	C	A
H42 secondary	B	E	C	D	A	A	BC	A
H72 secondary	C	E	BC	E	A	C	BC	B

[a] Statistical differences signified by different letters in the month columns; H4, high 4 day HRT; H42, high, secondary treatment 4 day HRT; H7, high 7 HRT; H72, high secondary treatment 7 day HRT; high refers to the inflow concentration treatment; Secondary, secondary P removal efficiency [1 – ($P_{secondary}$ – $P_{primary}$)]; System, system P removal efficiency [1 – ($P_{secondary}$ - P_{inflow}).

TABLE 3.5. Sequential P extractions of saturated calcined clay sampled from high 4- and 7-day HRT treatments. Values are the mean (standard error of the mean) of three sampling depths and four replicate tanks.

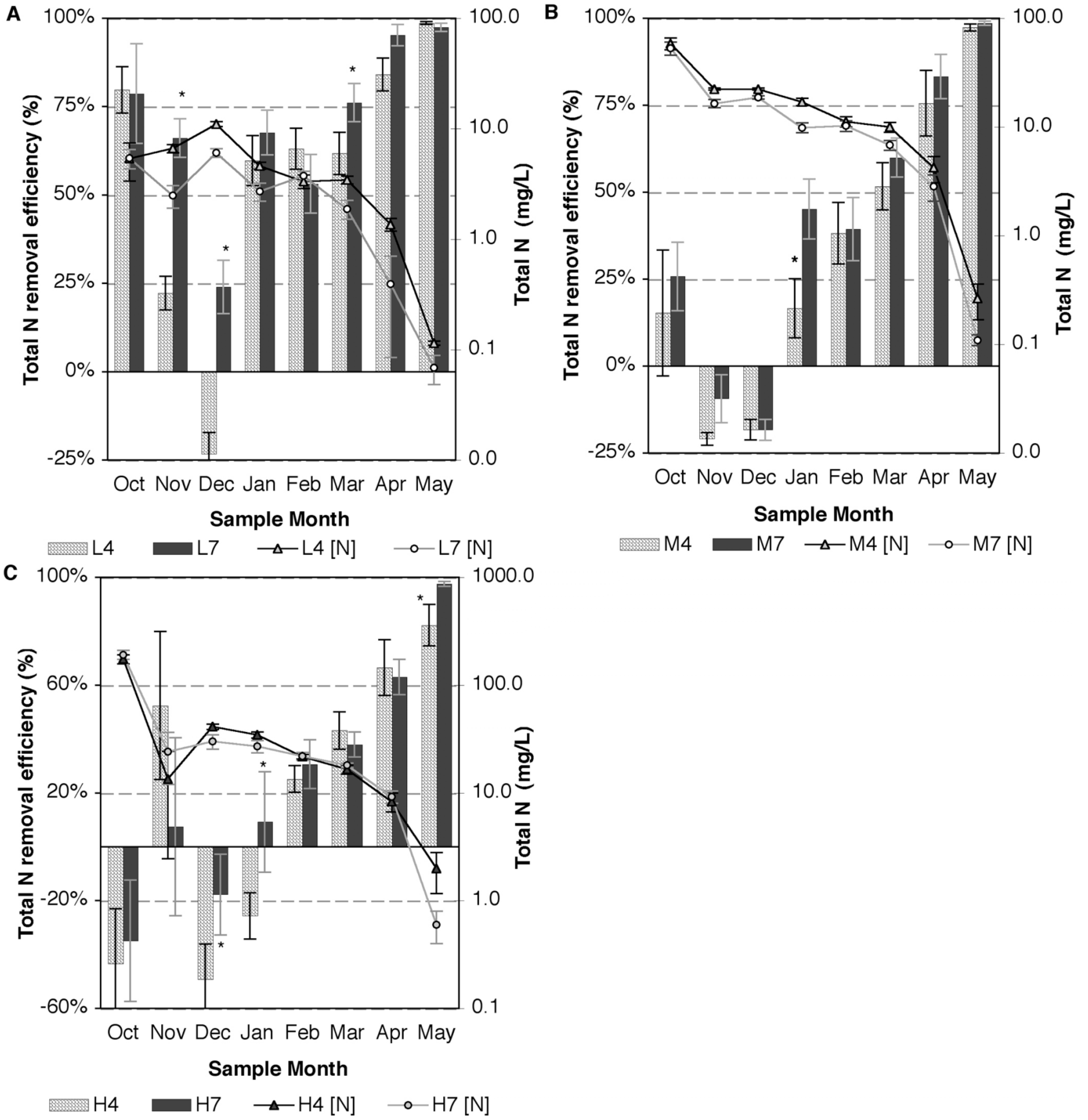
		Freely exchangeable	Plant available	Fe- and Al-extractable	Ca- and Mg-extractable	Residual	Total extracted
		mg P /kg substrate					
H42	Inflow	56.84 (3.66)	92.43 (13.67)	106.71 (22.59)	2068.39 (123.77)	116.93 (6.89)	2433.87 (115.93)
	Outflow	56.92 (5.15)	32.29 (3.58)	30.95 (2.49)	2189.55 (143.14)	125.55 (7.75)	2433.97 (147.74)
H72	Inflow	35.79 (1.80)	61.77 (6.39)	60.01 (9.65)	2286.21 (376.32)	130.05 (14.50)	2573.83 (397.37)
	Outflow	49.65 (3.84)	29.21 (4.97)	22.18 (4.03)	2188.29 (238.88)	129.56 (8.49)	2418.88 (246.37)

TABLE 3.6. Aluminum, iron, calcium, and magnesium concentrations extracted from P saturated calcined clay sampled from high 4- and 7-day HRT treatments. Values are the mean (standard error of the mean) of three sampling depths and four replicate tanks.

		Freely exchangeable	Plant available	Fe- and Al-extractable	Ca- and Mg-extractable	Residual	Total extracted
Aluminum (mg/kg)							
H42	Inflow	6.86 (0.57)	4.41 (0.39)	1146.11 (20.16)	4070.47 (115.38)	5639.27 (105.22)	10867.36 (162.25)
	Outflow	7.04 (0.81)	2.96 (0.47)	975.39 (13.45)	4250.54 (90.39)	5945.33 (121.39)	11181.18 (169.95)
H72	Inflow	6.93 (0.81)	0.69 (0.11)	960.50 (48.98))	4181.97 (114.25)	5874.94 (124.99)	11028.83 (194.83)
	Outflow	12.75 (1.90)	1.31 (0.40)	777.02 (29.30)	4249.82 (65.43)	6012.94 (101.76)	11050.05 (124.28)
Iron (mg/kg)							
H42	Inflow	7.62 (0.53)	22.96 (1.74)	14.28 (2.17)	3640.84 (58.38)	4593.74 (63.62)	8278.55 (106.48)
	Outflow	7.60 (1.29)	9.93 (0.40)	18.32 (1.64)	3690.17 (37.59)	4829.69 (63.59)	8555.39 (74.87)
H72	Inflow	10.06 (1.84)	14.87 (0.91)	8.81 (0.96)	3702.39 (61.82)	4841.45 (72.80)	8577.58 (119.61)
	Outflow	9.49 (1.15)	10.28 (0.95)	8.51 (1.26)	3709.07 (41.96)	4910.49 (56.78)	8647.84 (62.31)
Calcium (mg/kg)							
H42	Inflow	14.71 (1.41)	240.07 (22.82)	7.47 (1.21)	5708.61 (116.08)	451.31 (10.20)	6420.44 (123.84)
	Outflow	16.00 (2.73)	177.65 (15.13)	5.87 (1.25)	5805.99 (110.80)	464.44 (7.46)	6463.57 (112.58)
H72	Inflow	11.80 (2.19)	208.30 (16.23)	17.04 (2.94)	6754.94 (296.64)	490.00 (16.53)	7482.09 (314.59)
	Outflow	9.54 (1.04)	173.84 (20.30)	7.22 (1.66)	6109.02 (168.36)	516.56 (22.48)	6816.18 (173.99)
Magnesium (mg/kg)							
H42	Inflow	0.23 (0.07)	749.86 (33.15)	1.02 (0.19)	3933.05 (80.67)	4156.83 (73.07)	8840.96 (160.13)
	Outflow	0.18 (0.07)	1147.12 (25.02)	1.08 (0.16)	4298.16 (46.74)	4490.63 (81.35)	9882.92 (129.21)
H72	Inflow	0.44 (0.11)	750.88 (17.02)	1.27 (0.20)	4118.58 (79.50)	4367.39 (74.51)	9238.57 (111.70)
	Outflow	0.41 (0.08)	1042.46 (41.18)	0.71 (0.13)	4409.52 (57.00)	4640.28 (86.87)	10093.38 (112.25)

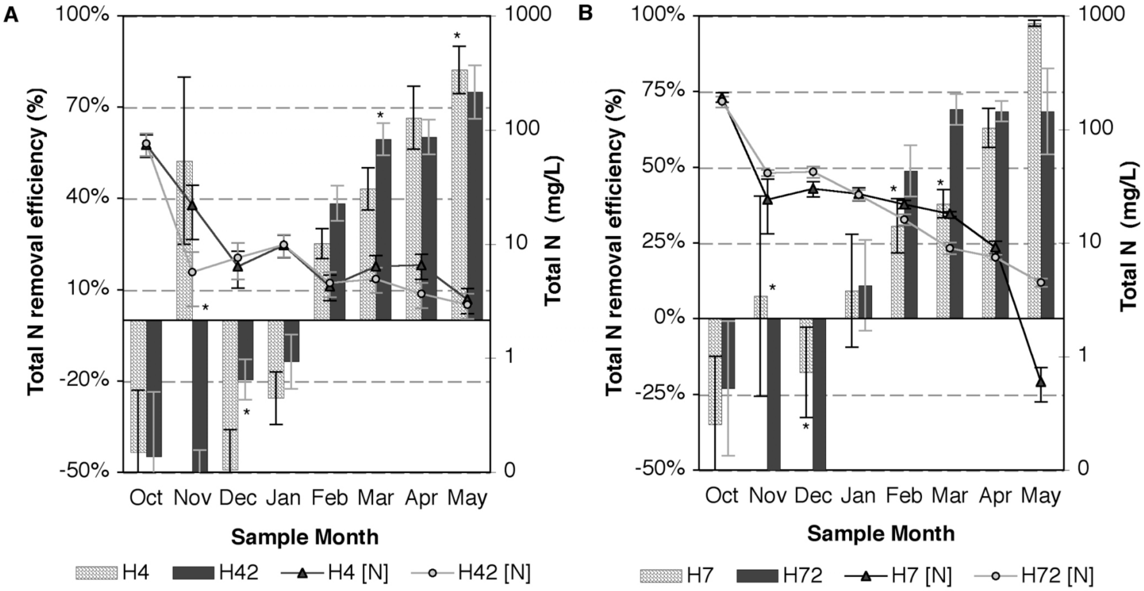
Figures

FIGURE 3.1. Total N ($NO_2 + NO_3 + NH_3$) removal efficiency (bars) and effluent concentration (lines) for primary (A) low, (B) medium, and (C) high nutrient inflow level treatments from October 2005 to May 2006. [a]



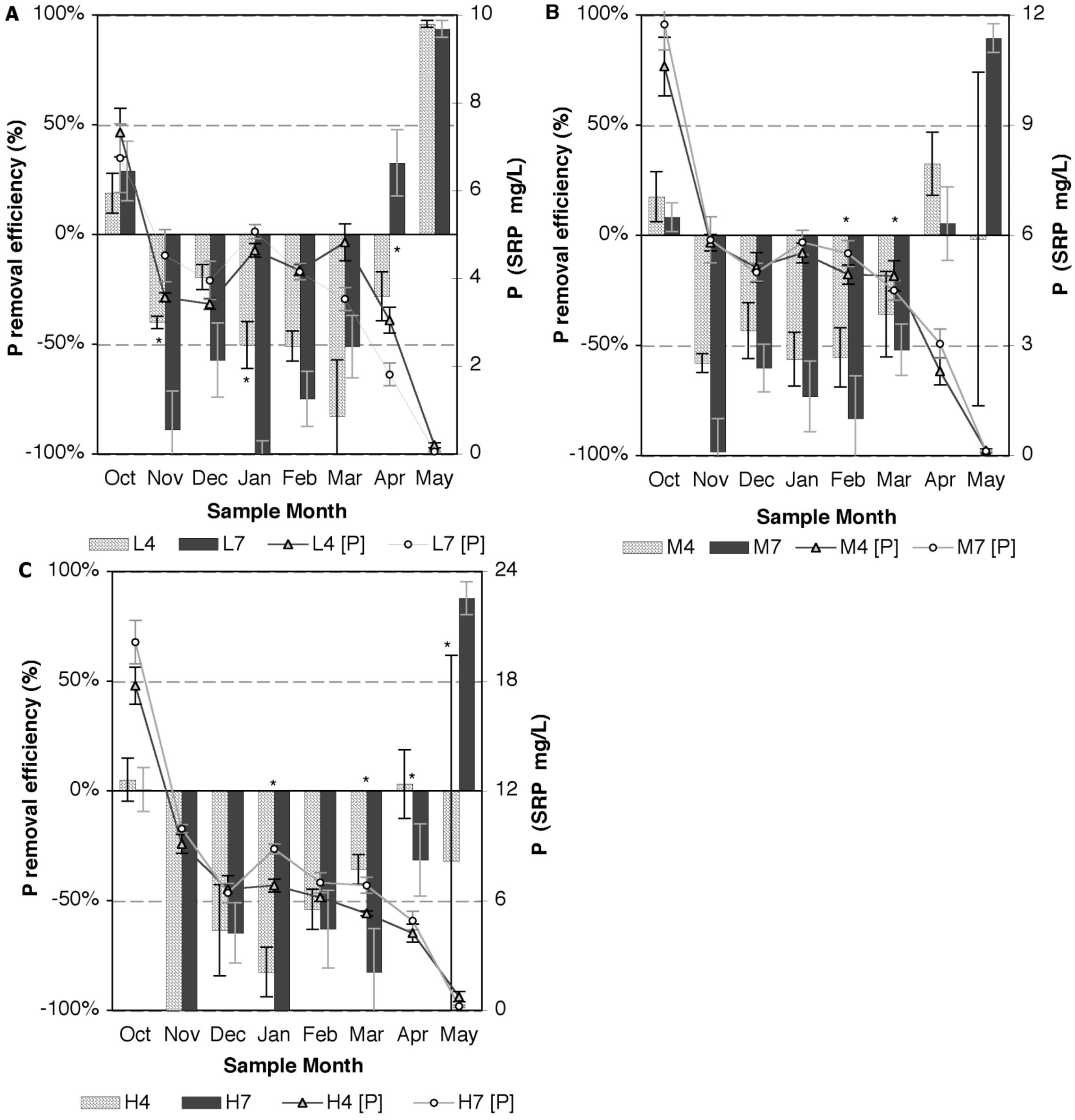
[a] Values represent averages of 4 replicates per sampling period ± standard error of the mean. Bars with * had statistically significant differences ($\alpha < 0.05$). L4, low 4 day HRT; L7, low 7 day HRT; M4, medium 4 day HRT; M7, medium 7 day HRT; H4, high 4 day HRT; H7, high 7 HRT.

FIGURE 3.2. Total N ($NO_2 + NO_3 + NH_3$) removal efficiency (bars) and export concentration (lines) with secondary subsurface-flow (A) High 4-day HRT and (B) High 7-day HRT mesocosm treatments from October 2005 to May 2006. [a]



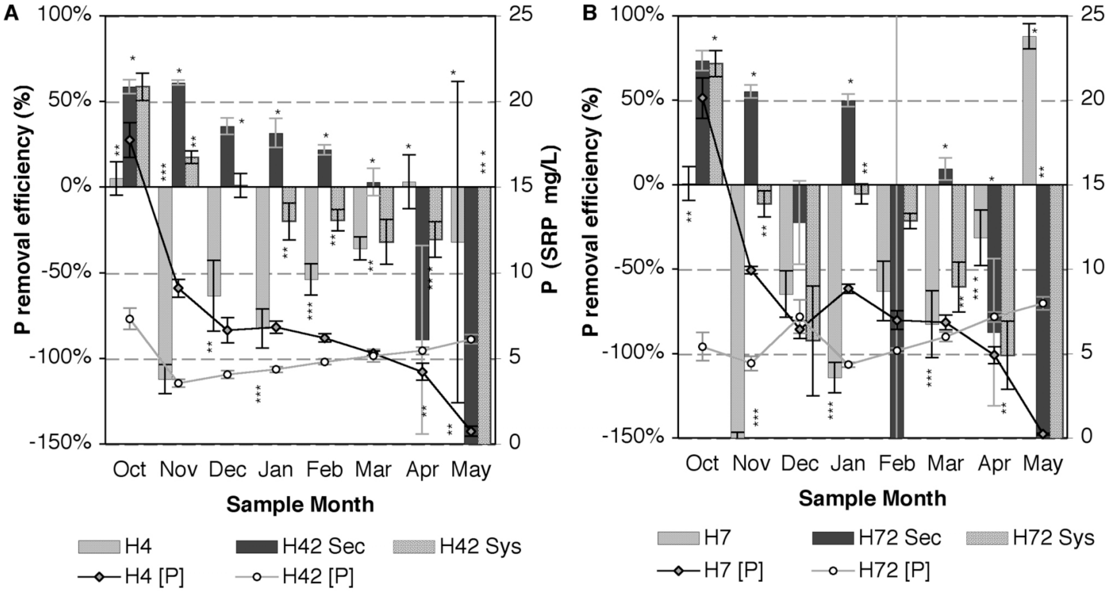
[a] Values represent averages of 4 replicates per sampling period ± standard error of the mean, with 4 sampling periods per month. Bars with * had statistically significant differences ($\alpha < 0.05$). H4, high 4 day HRT; H42, high, secondary treatment 4 day HRT; H7, high 7 HRT; H72, high secondary treatment 7 day HRT. High represents nutrient inflow level treatment.

FIGURE 3.3. Soluble reactive phosphorus removal efficiency (bars[a]) and export concentrations (lines) by primary mesocosm (A) low, (B) medium, and (C) high treatments from October 2005 to May 2006.



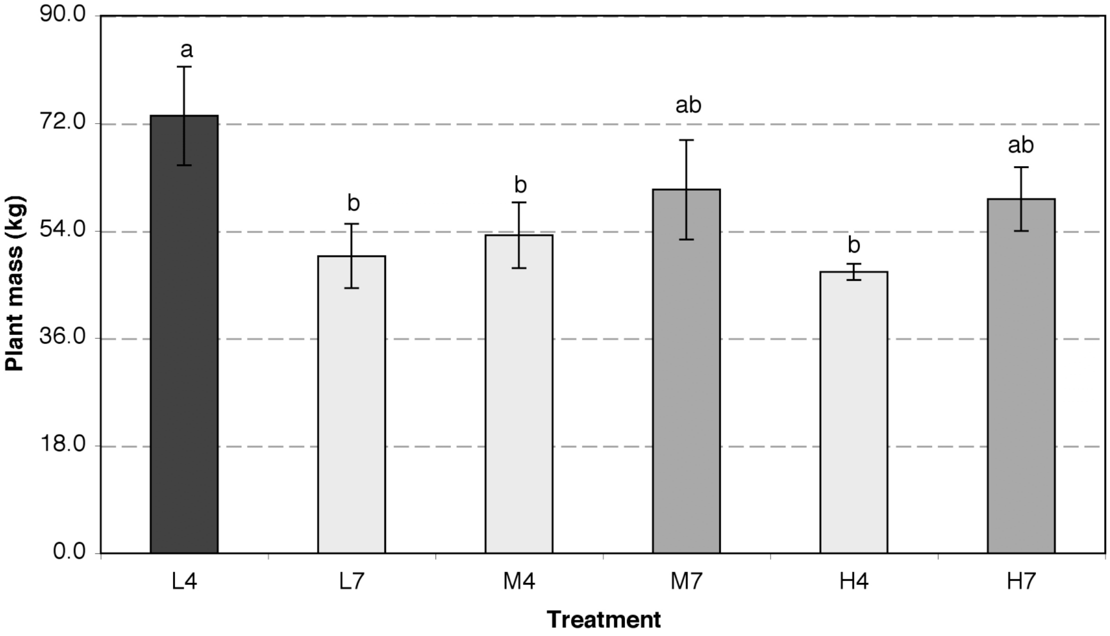
[a] Values represent averages of 4 replicates per sampling period ± standard error of the mean, with 4 sampling periods per month. Bars with * had statistically significant differences ($\alpha < 0.05$). L4, low 4 day HRT; L7, low 7 day HRT; M4, medium 4 day HRT; M7, medium 7 day HRT; H4, high 4 day HRT; H7, high 7 HRT. Low, medium, and high are nutrient inflow level treatments.

FIGURE 3.4. Soluble reactive phosphorus removal efficiencies (bars) and export concentrations (lines) of primary surface-flow to secondary subsurface-flow (A) High 4-day HRT and (B) High 7-day HRT mesocosm treatments from October 2005 to May 2006. [a]



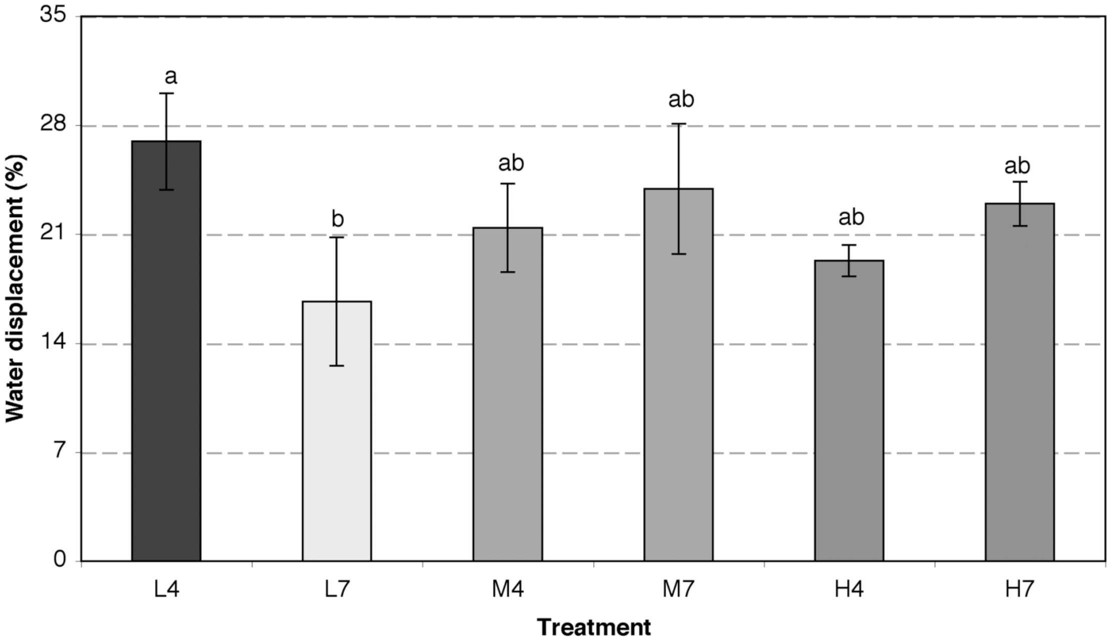
[a] Values represent averages of 4 replicates per sampling period ± standard error of the mean, with 4 sampling periods per month. Bars with * had statistically significant differences ($\alpha < 0.05$). H4, high 4 day HRT; H42, high, secondary treatment 4 day HRT; H7, high 7 HRT; H72, high secondary treatment 7 day HRT; Sec., Secondary P removal efficiency [1 – ($P_{secondary} – P_{primary}$)]; Sys., System P removal efficiency [1 – ($P_{secondary}$ - P_{inflow}).

FIGURE 3.5. Macrophyte biomass (shoots and roots) at harvest from mesocosms. [a]



[a] Values represent averages of 4 replicates per treatment ± standard error of the mean. Bars with different letters were significantly different ($\alpha < 0.05$). H4, high treatment 4 day HRT; H7, high treatment 7 day HRT; M4, medium treatment 4 day HRT; M7, medium treatment 7day HRT; L4, low treatment 4 day HRT; L7, low treatment 7 day HRT. Low, medium, and high are nutrient inflow level treatments.

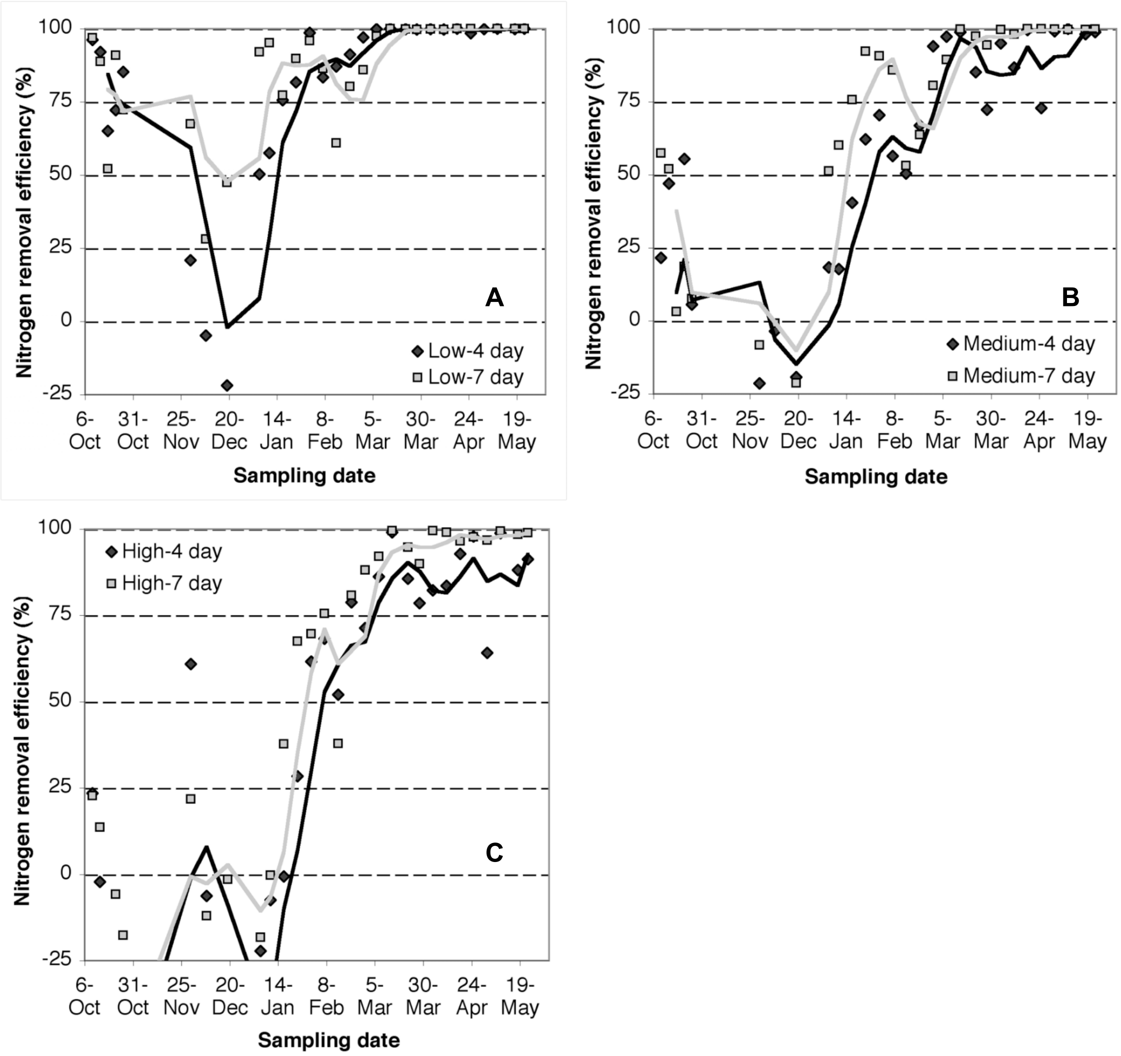
FIGURE 3.6. Macrophyte biomass percent displacement of water in mesocosms. [a]



[a] Values represent averages of 4 replicates per treatment ± standard error of the mean. Bars with different letters were significantly different ($\alpha < 0.05$). H4, high treatment 4 day HRT; H7, high treatment 7 day HRT; M4, medium treatment 4 day HRT; M7, medium treatment 7day HRT; L4, low treatment 4 day HRT; L7, low treatment 7 day HRT. Low, medium, and high are nutrient inflow level treatments.

Supplementary Figures

FIGURE S3.1. Nitrogen removal efficiency of primary, surface-flow mesocosms for all sampling dates from October 2005 to May 2006. (A) Shows Low[a] 4- and 7-day treatment removal efficiency, (B) shows Medium 4- and 7-day treatment removal efficiency; and (C) shows High 4- and 7-day treatment removal efficiency.



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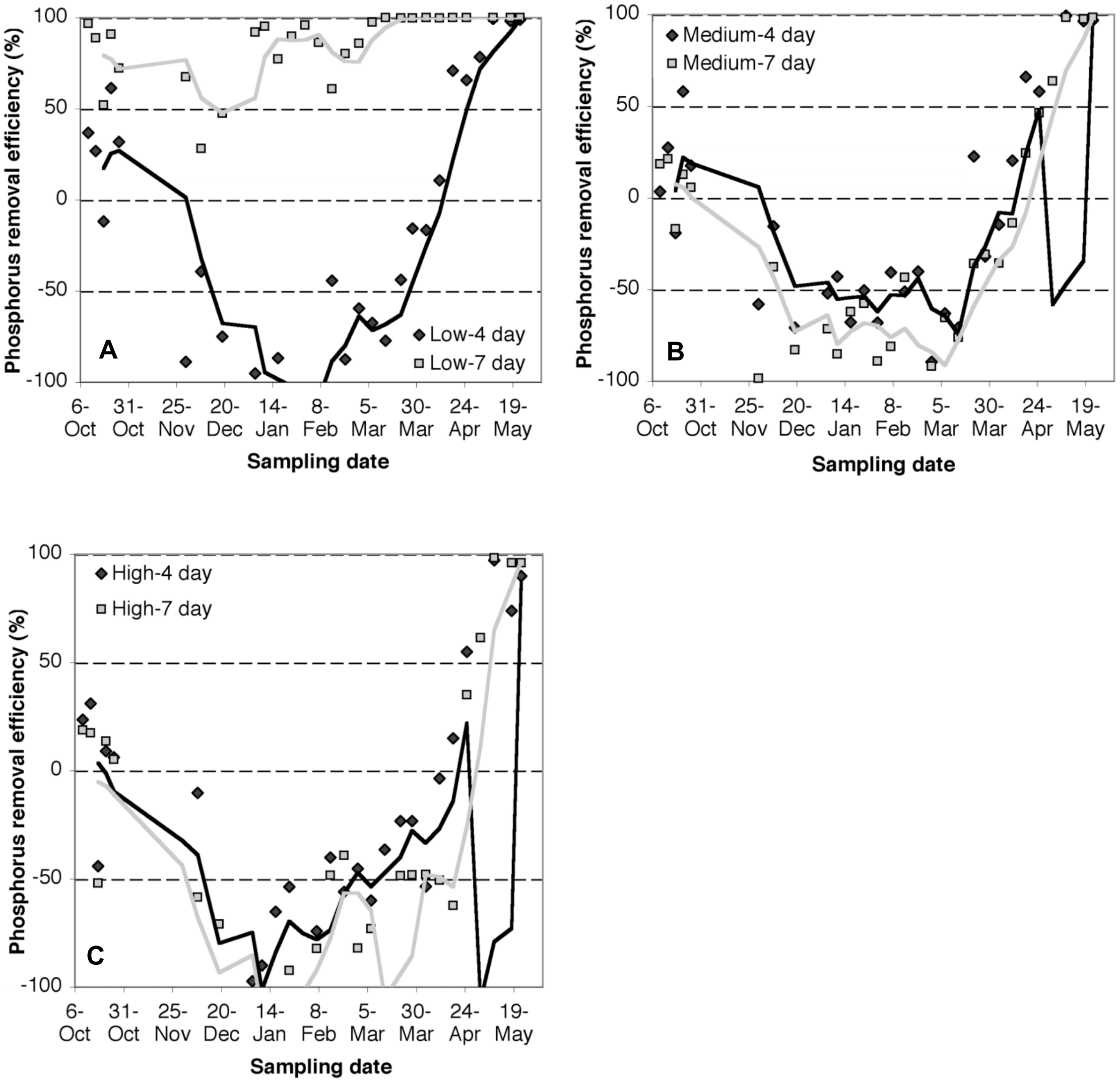
[a] Moving average trendlines for three sampling dates fitted to track N removal efficiencies. Low, medium, and high are nutrient inflow level treatments.

FIGURE S3.2. Phosphorus removal efficiency[a] of primary, surface-flow mesocosms for all sampling dates from October 2005 to May 2006. (A) Shows Low[a] 4- and 7-day treatment removal efficiency, (B) shows Medium 4- and 7-day treatment removal efficiency; and (C) shows High 4- and 7-day treatment removal efficiency.



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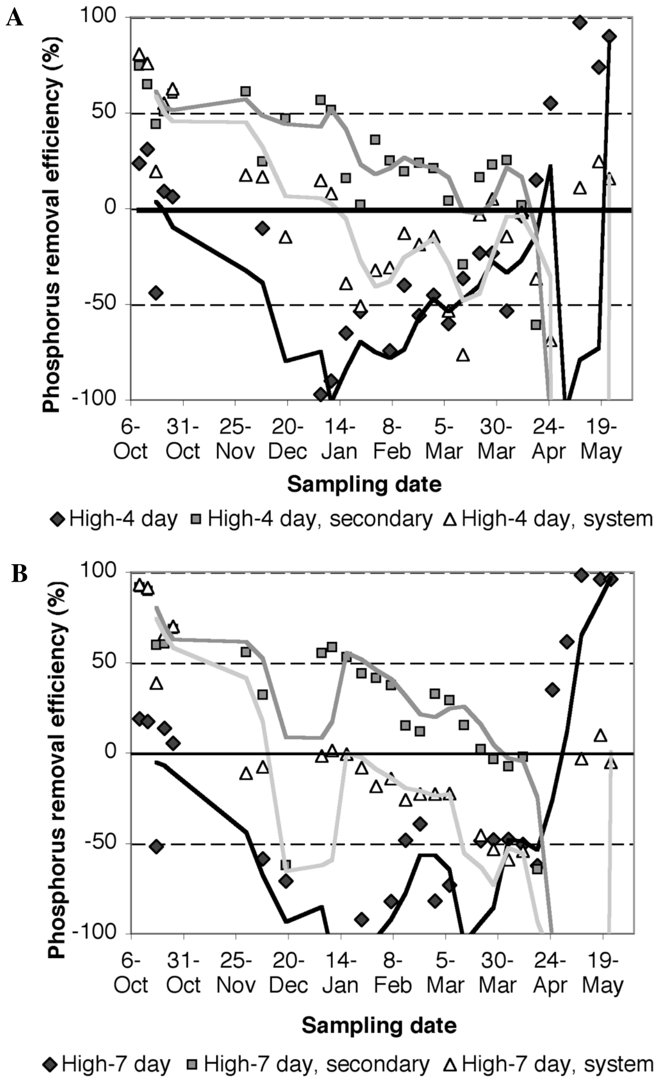
[a] Moving average trendlines for three sampling dates fitted to track P removal efficiencies. Low, medium, and high are nutrient inflow level treatments.

FIGURE S3.3. Phosphorus removal efficiency of primary surface-flow to secondary sub-surface flow mesocosms for all sampling dates from October 2005 to May 2006. (A) Shows High[a] 4-day secondary and system P removal efficiency, (B) shows High 7-day secondary and system P removal efficiency.



[a] Moving average trendlines for three sampling dates fitted to track P removal efficiencies. Secondary, P removal efficiency [$1 - (P_{secondary} - P_{primary})$]; System, P removal efficiency [$1 - (P_{secondary} - P_{inflow}$).

FIGURE S3.4. Average water temperature (ºC) from October 2005 to May 2006. Each data point is the mean of 24 mesocosms ± the standard deviation of the mean.

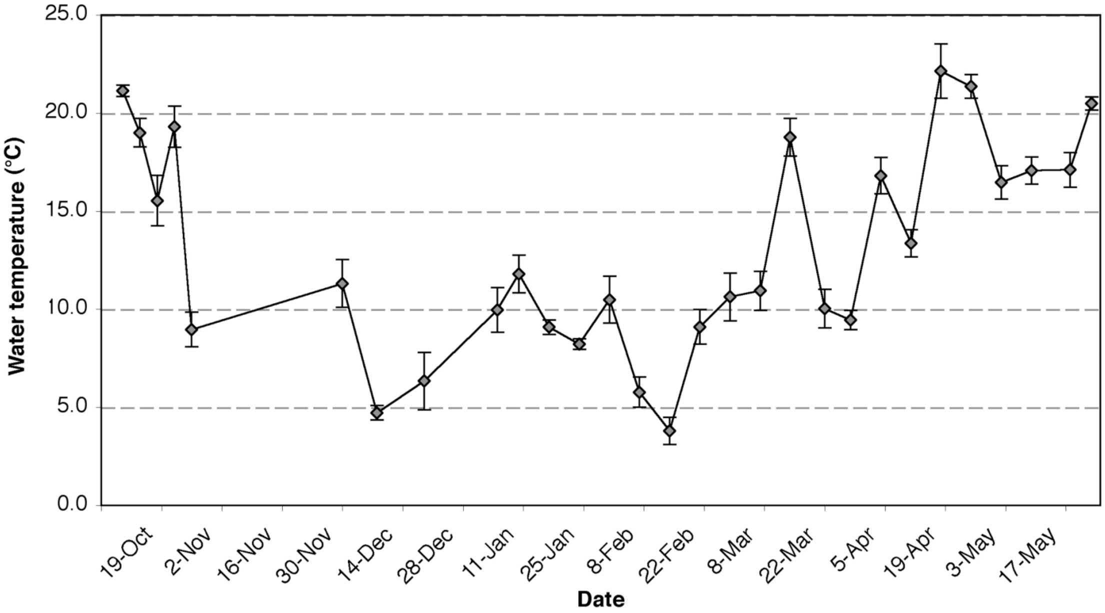
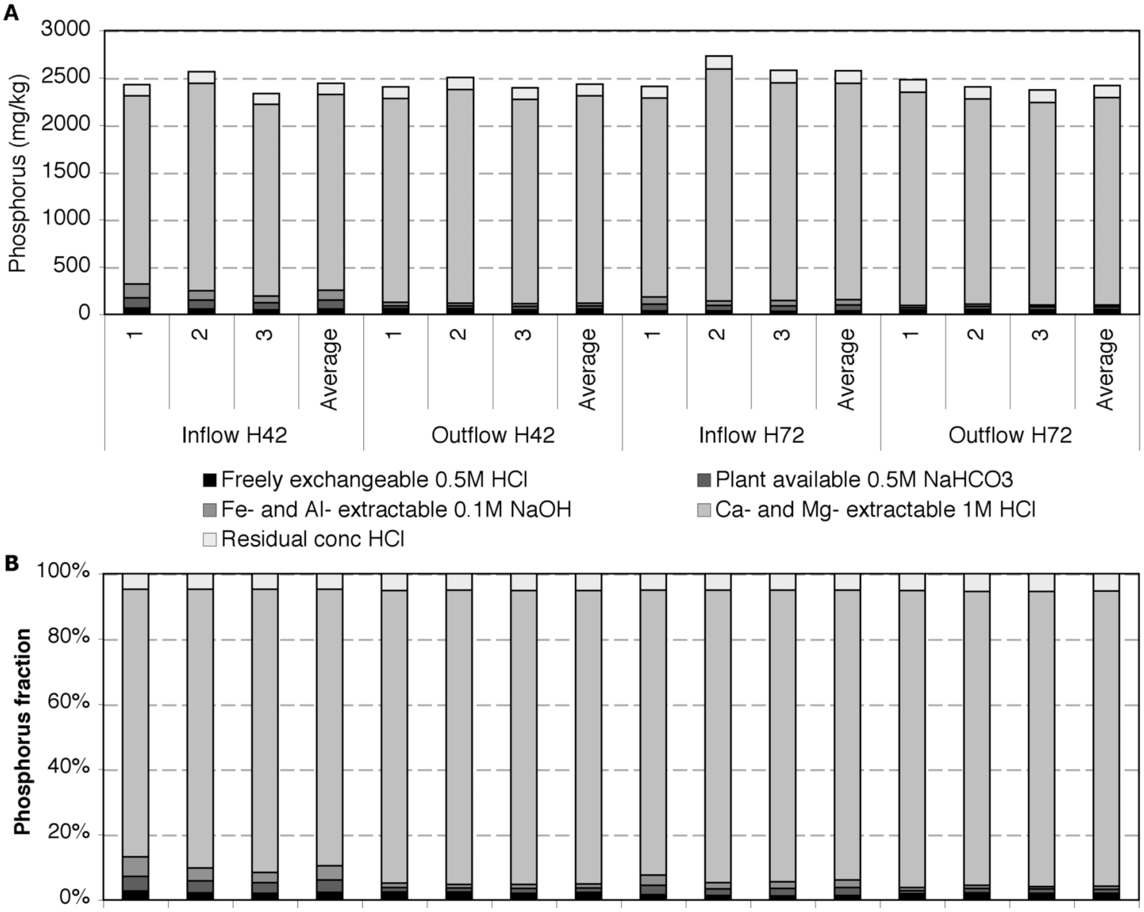


FIGURE S3.5. Sequential P extractions of saturated calcined clay sampled at three depths (1 to 3) and two locations (inflow and outflow) from secondary subsurface-flow mesocosms[a]; A) concentration of P from each extraction step, and B) percent of total P extracted represented by each extraction step.



[a]Values are the mean of two samples taken at each depth from four mesocosms for each treatment: H42, high inflow concentration and 4-day hydraulic retention time (HRT); and H72, high inflow concentration and 7-day HRT. Depth 1 is top 3 cm of CC, 2 is the middle layer, and 3 is the bottom layer.

FIGURE S3.6. Residuals of total nitrogen plotted as a function of residuals of various regression parameters.

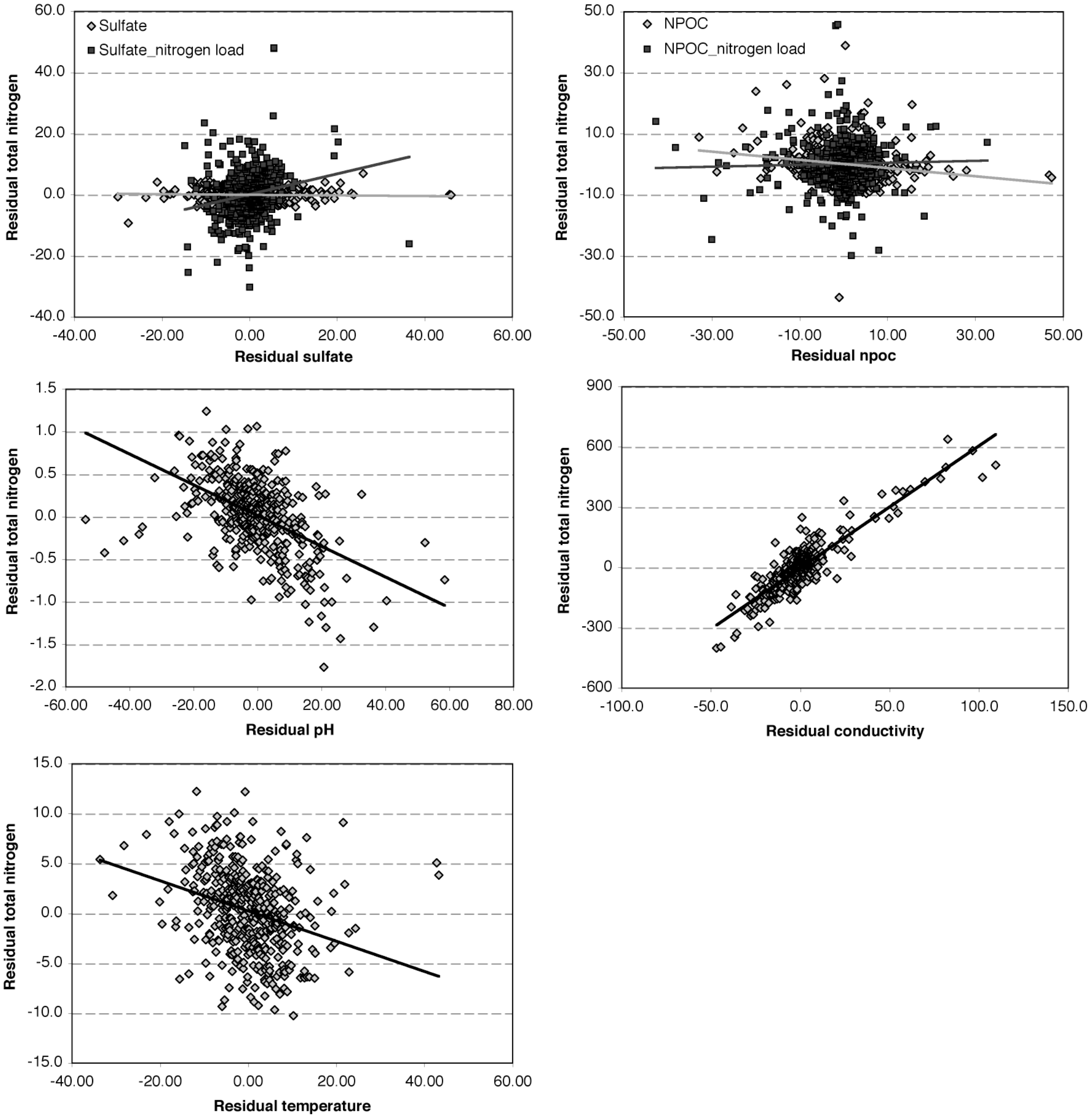
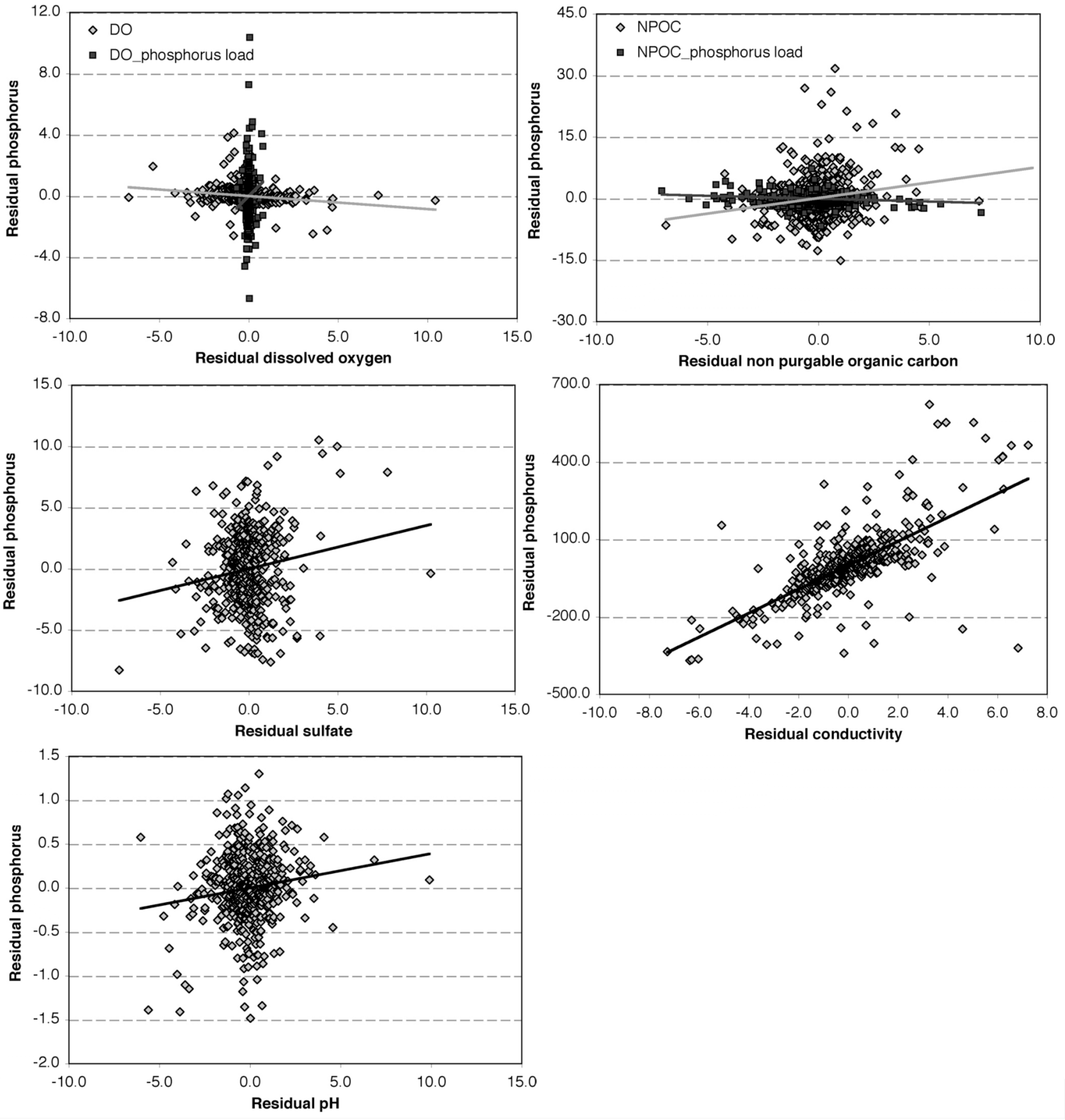


FIGURE S3.7. Residuals of phosphorus plotted as a function of residuals of various regression parameters.



CHAPTER 4: NURSERY NUTRIENT RUNOFF CONCENTRATIONS REDUCED BY FLOATING-MATS AND FIRED CLAY SUBSTRATES IN SURFACE- TO SUBSURFACE-FLOW CONSTRUCTED WETLANDS

Abstract

Nutrient rich runoff from nursery operation production areas can potentially impair surface water quality offsite. Constructed wetland systems (CWS) are low maintenance treatment systems that can reduce nutrient export. This investigation examined surface (primary) to subsurface (secondary) flow CWS for nutrient removal efficiency. The primary mesocosm treatment was established with three planting types: 1) Floating mats of typical wetland plants, 2) wetland plants rooted into substrate, and 3) horticultural cultivars selected for nutrient uptake ability planted as a mixture of floating mat and rooted in substrate plants. Secondary mesocosms were established with brick or industrial mineral aggregate and then were either planted or unplanted. The floating treatment attained the highest nitrogen removal efficiency (NRE, 95.5 ± 0.5 %), and the rooted treatment facilitated the greatest phosphorus removal efficiency (PRE, 78.9 ± 1.1 %). Horticultural cultivars were least efficient in promoting both NRE and PRE. Planted secondary treatment NRE (98.1 ± 0.9 %) and PRE (94.9 ± 1.2 %) was consistently greater than the NRE (90.6 ± 0.4 %) and PRE (91.9 ± 1.7 %) of the unplanted treatment. Unplanted industrial mineral aggregate sorbed P more efficiently than the brick, resulting in lower effluent concentrations, 0.24 ± 0.05 mg/L compared to 0.57 ± 0.11 mg/L.

Planted secondary treatment reduced P export further to 0.13 ± 0.02 and 0.37 ± 0.10 mg/L P for industrial mineral aggregate and brick, respectively. Mixed surface to subsurface flow CWS were highly effective at reducing nutrient export.

Introduction

The greenhouse/nursery industry is one of the fastest growing segments of agriculture in the United States. Nursery operations with \$100,000 or more in sales were surveyed in 17 states and total gross sales were \$4.65 billion in 2006, an increase of 17% over three years (1). Over 471,106 acres are in production by nurseries with sales over \$10,000. Larger operations are able to produce high quality plants in a relatively short period of time by optimal application of nutrients, pesticides, and irrigation (2). Some large nurseries utilize > 37,850 kL fresh water per day for irrigation (1). Large-scale application of nutrients, pesticides, and water to containerized plant production beds often results in very large quantities of runoff containing pesticides and substantial nutrient concentrations (2-4).

An increased knowledge of surface water quality impairment from excess nutrient loading has raised awareness in the public, private, and governmental sectors resulting in the enforcement of regulations limiting nutrient runoff from nursery operations in Australia and in a number of states in the United States, e.g., California, Delaware, Florida, Maryland, and Oregon (2, 5-6). The U.S. Environmental Protection Agency (EPA) has mandated maximum allowable NO_3-N contaminant levels in any discharged water of $\leq$10 mg/L to protect drinking water quality, and, while no federal limits for P concentrations leaching into freshwater have been set, the EPA recommends that total inorganic phosphorus not exceed 0.05 mg/L and total P (inorganic + organic forms) not exceed 0.1 mg/L (7).

Nursery operations have several management strategies available to manage runoff including denitrification walls and vegetated/turfgrass buffer strips for low flow volumes (8-11), vegetated ditches for low to moderate volumes (12), and constructed wetlands for moderate to high volumes of runoff (3-4). Remediation system choice depends on runoff volume and frequency, whether continuous flow or intermittent, and nutrient loading in runoff. Constructed wetland systems offer a low-tech, low maintenance remediation strategy for runoff either for release or recycling. Both surface- and subsurface-flow CWS are effective at nutrient removal (3-5, 13-17). Typically, CWS have been established with plant monocultures of *Phragmites australis* (common reed), *Typha* spp. (cattail), and *Scirpus* spp. and *Schoenoplectus* spp. (bulrushes) (5, 13-17). Only in recent years have researchers begun to examine the role of mixed macrophytes in CWS for nutrient remediation. In some cases, plants with high nutrient uptake capacities also release those nutrients very quickly upon senescence while other plants retain the assimilated nutrients (18). Some plant species assimilate nutrients at low exposure concentrations and others only efficiently remove N and P with higher exposure concentrations (19). Mixed species studies have shown increased macrophyte richness in CWS improves wetland function and nutrient processing capacities (4, 11, 15, 18, 20-22). Therefore, CWS installation strategies incorporating those plant species with large nutrient assimilation capacities at high nutrient concentrations near inflow pipes, and nutrient ‘miners,’ which efficiently assimilate nutrients at lower concentrations near outflow pipes, would combine the best of both nutrient uptake strategies, and could result in increased nutrient removal efficiency.

While surface-flow wetland systems generally facilitate high nitrogen removal efficiency (NRE), seasonal shifts in NRE have been noted (3, 21-25). This seasonal remediation shift occurs during winter months when plant growth and nutrient uptake are reduced, and when water temperatures decrease to the point where biological processes are inhibited (22 - 25). However, phosphorus removal efficiency (PRE) is not subject to temperature dependence; instead PRE is controlled to varying extents by plant uptake, sedimentation, microbial immobilization, precipitation, and sorption to root bed media (26-29). The P sorption potential of root-bed media in subsurface-flow CWS is very important. Typically, higher sorption capacities facilitate greater P sorption and the type of substrate present or used can influence the length of time that P is sorbed. Many studies have examined a range of P sorbing substrates (gravel, dolomite, furnace slag, fly ash, shale, limestone, oyster shells and sand) and shown varying degrees of P sorption and removal efficiencies ranging from 10 to 71% (28, 30 - 32).

The objectives of this study were 1) to characterize the effect of CWS establishment type on nutrient removal efficiency by examining nutrient attenuation differences among mesocosms initially established with floating mats, root incorporation into substrate, or a mixture of the two, utilizing horticultural plants selected for their N and P uptake capacities; 2) to evaluate the relative PRE and longevity of two clay-based substrates in subsurface-flow mesocosms; and 3) to compare nutrient removal efficiencies of vegetated and non-vegetated subsurface-flow clay treatments.

Methods

Surface to Subsurface-Flow Mesocosm

A two-stage surface to subsurface-flow wetland design was used to maximize both nitrogen and phosphorus remediation efficiency (Figs. S4.1 and S4.2). The primary stage, surface-flow mesocosms utilized twenty-four 380-L stock tanks established with one of three planting-type treatments, 1) floating, 2) selected cultivars, or 3) rooted treatments. The floating treatment consisted of plants typical of natural wetlands/bogs and was established as a floating mat. The rooted treatment consisted of plants typical of natural wetlands/bogs that were established via planting into gravel substrate. The horticultural cultivars were chosen from a set of species screened by Polomski et al. (19) as plants with high nutrient recovery rates, and were established as a mixture of floating mat and substrate rooted plants.

The bottom of each mesocosm was lined with approximately 15.2 cm of granite pea gravel. Plants were either established as floating mats, planted in the gravel, or a mixture of both types. Water levels were raised slowly to maximum fill level. Mesocosms were planted in August 2006 and allowed to establish for nine months before sampling began on 11 May 2007 and sampling continued through 8 October 2007. The establishment period was longer because research has shown that in CWS more than one season may be needed to establish "natural wetland conditions" (15, 33). Thus sampling started during the second growing season. Hydraulic retention time was four days with a flow rate of 67.8 ± 5.01 L/day.

Each secondary, subsurface-flow mesocosm received discharge piped from its corresponding primary mesocosm (Fig S4.2). Twenty-four secondary mesocosms, 190 L stock tanks, were filled with approximately 90.7 kg of a coarse (0.8 to 4.75 mm) industrial mineral aggregate [calcined clay (CC), Oil-dri Corp. of America, Chicago] or coarse crushed brick (0.8 to 4.57 mm, National Brick Research Institute, Clemson, SC). Six of the CC and six of the brick secondary mesocosms were vegetated with horticultural cultivars, while 12 remained unplanted (six of each). The horticultural cultivars were planted into the secondary mesocosms on 11 May 2007, after all secondary mesocosms were filled with substrate. Secondary treatments were not filled with substrate until one week prior to sampling initiation.

Planting Type

Approximately 13.4 ± 4.7 kg of plant material were added to each of the eight floating-type primary mesocosms. Typical wetland species incorporated into floating treatment mesocosms were *Sagittaria latifolia* (arrow-head), *Hydrocotyle umbellata* (marsh pennywort), *H. ranunculoides*, (water pennywort), *Pontaderia cordata* (pickerelweed), and *Canna flaccida* (Florida canna). The eight rooted-type, primary mesocosms were established with approximately 12.1 ± 4.7 kg of plant material and wetland species included were pickerelweed, arrow-head, *S. graminea* (duck potato), Florida canna, and *Panicum hemitomum* (maidencane).

Eight primary mesocosms were planted with horticulturally-important cultivar (plants of importance to the green industry) treatments, and 11.9 ± 5.8 kg of plant material were added to each. Species planted were *Juncus effusus* var. *effusus* (soft rush), *Thalia geniculata* (thalia), *Iris* x *louisiana* ‘Full Eclipse’, *I. neomarica caerulea* '*Regina*' (iris Regina), *I. louisiana* '*Yellow*' (yellow flag iris), *Canna* 'Australia', *Itea virginica* 'Henry's Garnet' (Virginia sweetspire), *Crinum americanum* (swamp lily), and *Eleocharis palustris* (water chestnut).

Horticultural cultivars planted in secondary, subsurface-flow mesocosms were the following: *Iris neomarica caerulea* ‘Regina’, *Carex laxiculmis* ‘Hobb Bunny Blue’ (Hobb bunny blue sedge), *Carex plantaginea* (seersucker sedge), *Canna* ‘Paton’, *Canna* ‘Intrigue’, *Typha minima* (dwarf cattail), *Acorus gramineus* ‘Dwarf Green’ (dwarf sweet flag), *Alocasia wentii* (hardy elephant ear), *Colocasia antiquorum* ‘Black Beauty’ (black beauty elephant ear), and *Iris louisiana.*

Simulated Nursery and Greenhouse Runoff

Mesocosms received nutrients from solutions pumped from 1135.6 L holding tanks. The simulated nursery and greenhouse runoff was prepared using a 20-2-20 nitrate special commercial-grade water-soluble fertilizer (Southern Agricultural Insecticides Inc., Hendersonville, NC) and a 0-52-34 phosphate special water-soluble fertilizer (Haifa Chemicals, Israel). The nutrient concentration supplied to mesocosms was 10.70 ± 2.71 mg/L N and 4.98 ± 0.42 mg/L P.

Sampling Endpoints

Water samples were taken weekly and analyzed for NH_3^+, NO_3^-, NO_2^-, PO_4, pH, total organic carbon (TOC), and total dissolved nitrogen (TN), and water temperature (°C) was recorded. Anions were determined using a Dionex AS10 ion chromatograph with AS50 auto-sampler (Dionex Corp., Sunnyvale, CA). TOC and TN were determined using a Shimadzu TOC-V $_{CPH}$ total organic carbon analyzer with TNM-1 total nitrogen measuring unit (Shimadzu Scientific Instruments, Kyoto, Japan). Ammonia was measured using an Orion Ammonia Electrode 95-12 (Thermo Electron Corp., Beverly, MA), and pH was measured using an Orion 710A+ pH meter.

Statistical Analyses

Treatment effects were analyzed using SAS PROC GLM with LSD means separation (SAS Institute Inc., Cary, NC).

Results and discussion

Nitrogen and phosphorus removal efficiency in primary mesocosms

Primary mesocosms were established with different planting types to differentiate the influence of planting type and to determine if there were long-term impacts on nutrient remediation efficiency from planting type. Total N (TN, $NO_3 + NO_2 + NH_3$) concentrations were monitored over the 5 months of this study and remediation efficiencies calculated (Fig. 4.1). Freezing temperatures two weeks before sampling initiation may have contributed to the lower nitrogen removal efficiency (NRE) of the horticultural-cultivar treatment, while the native wetland plant species in the floating-mat and rooted treatments were not as sensitive to freeze damage. By early June, all treatments were functioning with NREs > 90%, resulting in outflow TN concentrations < 1 mg/L TN.

All treatment NREs were similar and highly efficient until the last week in July. At this point, average water temperatures increased beyond 28 ºC (Fig S4.3). This higher water temperature may have temporarily decreased microbial growth and processing, but after a period of acclimation, N assimilation by microbes increased, resulting in TN export of < 1.0 mg/L for rooted and floating treatments. Throughout the remaining sampling dates, the horticultural-cultivar treatment was less efficient than either the rooted or floating-mat treatment. The floating-mat treatment consistently delivered the highest NRE, exporting only 4.4% of the TN loaded. Vymazal (26) has reported that free-floating plants also achieve high NRE. The increased NRE of the floating-mat treatment may be attributed to two factors: 1) the plant mix used to establish the treatments and 2) the greater root surface area in the water column of the floating treatment compared with the rooted treatment. Nitrogen assimilation over the study period was 1,913 ± 7.6 g (97.3 ± 0.2 %) by the floating-mat treatment, 1,838.6 ± 7.1 g (93.5 ± 0.3 %) by the rooted treatment, and 1,703 ± 7.3 g (86.6 ± 0.4%) by horticultural-cultivar treatment (Table 4.1).

Phosphorus removal efficiency (PRE) in the primary mesocosms was highly variable compared to NRE. However, wetland aided phosphorus removal is inherently variable (4) and does not correlate with water temperature or season as does N removal (22). Primary treatments maintained positive PRE (Fig 4.2), and few significant treatment effects were found consistently after May. The rooted treatment facilitated the most consistent PRE throughout the experiment. However, both horticultural-cultivar and floating-mat treatments facilitated similar PREs to the rooted treatment after May, averaging 69.4 ± 8.3 and 76.0 ± 6.8 % respectively, while PRE by the rooted treatment averaged 81.3 ± 0.3 %. These removal efficiencies were relatively high for the summer as some studies have shown higher PRE in spring before attainment of maximum growth by surface-flow constructed wetlands, followed by a decline in PRE during the summer when active plant growth slows (4, 26). However, Picard et al. (22) noted consistent PRE from subsurface-flow microcosms from March through October followed by consistent export from November through February.

During September and October, the rooted treatment had consistently higher PRE, even though no significant treatment effects were detected (Fig. 4.2). Rooted treatment outflow concentration was 0.92 ± 0.20 mg/L P, while floating-mat and horticultural-cultivar treatment effluent P concentrations averaged 1.76 ± 0.46 mg/L. Even though no statistical differences were detected among treatments from September to October, the two-fold increase in P export concentration between the rooted treatment and the floating-mat and horticultural-cultivar treatments was very significant, biologically speaking. An additional export of 1 mg/L P from these treatments could have dramatic ecological effects downstream, since only minor (0.01 to 0.05 mg/L) increases in P in effluent have been shown to contribute to increased eutrophication rates (4, 7).

The horticultural-cultivar treatment was consistently less efficient at nutrient removal at lower nutrient inflow concentrations (~ 10.7 mg/L N). Research by Polomski et al. (19) suggested *Canna* and *Iris* might be especially effective at nutrient assimilation in CWS. However, *Canna* assimilated N and P most efficiently when exposure concentrations were > 21.57 mg/L N and > 3.63 P (19). Since, the loading rate of N into the mesocosms never reached this concentration, *Canna* assimilation efficiency may not have been at its peak. *Iris* was most efficient at assimilating nutrients when N and P exposure concentrations were < 15.0 mg/L (19) as were many of the other plant species used to establish these mesocosm. However, it appears that some condition(s) was not met to promote the maximal assimilative capacities of these horticultural-cultivars.

Native wetland/bog plants in both rooted (higher PRE) and floating-mat (higher NRE) treatments demonstrated highly efficient nutrient assimilation at nutrient inflow rates used in this experiment. These results suggest that CWS installation incorporating a mixed planting strategy, with both rooted plants and floating mats, may provide optimized N and P removal efficiencies.

Nitrogen and phosphorus removal efficiency in secondary mesocosms

The secondary subsurface-flow treatment mesocosms were filled with either coarse CC or brick. Half were planted with horticultural cultivars, while the other half remained unplanted. The clay and brick, vegetated (veg.) and non-vegetated (nonveg.) treatments were assigned randomly to receive effluent from primary treatments. The secondary treatments were targeted at reducing TP in effluent and not for further reduction in TN. However, it is important to characterize the N assimilation potential in the secondary treatment as well.

Calcined clay and brick substrates were ineffective at further reducing TN (Figs. 4.3 and 4.4); instead, they released additional N (average concentration 1.0 ± 0.15 mg/L TN) to the effluent. These TN concentrations, though relatively low, resulted in highly negative secondary removal efficiencies, even though system (SYS) efficiencies remained positive (Fig. 4.3b and 4.4b). Secondary (SEC) removal efficiency was calculated as the difference in nutrient concentration between primary outflow and secondary treatment outflow, and SYS removal efficiency was calculated as the difference in nutrient concentration between the initial inflow concentration and secondary treatment outflow concentration.

Nitrogen removal efficiency was much higher for planted secondary treatments compared to unplanted, substrate-filled treatments. This additional TN fixation could be due to plant-mediated uptake, because secondary treatments with substrate alone resulted in decreased NRE. Total nitrogen fixation credited to plant uptake was 119.1 ± 3.3 g TN for the brick treatment and 141.1 ± 3.0 g for the CC treatment (Table 4.1). Other researchers have found similar patterns with veg. and nonveg. microcosms (15) from carbon limitation on denitrification in the nonveg. mesocosms and the additional N uptake by plants (34). Mesocosm NPOC concentrations were not significantly different among the veg. and nonveg. secondary treatments (Fig. S4.4). Consequently, plant uptake and not NPOC limitation may have controlled the additional NRE in the veg. treatment mesocosms.

Phosphorus removal efficiency in the secondary treatment mesocosms was relatively steady (Figs. 4.5A and 4.6A). Throughout the experiment, SYS CC-veg. and CC-nonveg. treatments did not differ significantly. However, CC-veg. treatment PRE was consistently slightly higher than CC-nonveg. treatment PRE. An additional 166.9 ± 4.4 and 209.6 ± 3.4 g of P were fixed by the CC-nonveg. and CC-veg. treatments respectively (Table 4.1). The CC treatment was very efficient and reduced secondary effluent concentration to 0.24 ± 0.05 mg/L for nonveg. and 0.13 ± 0.02 mg/L P for veg. mesocosms. Although P export concentration from the CC treatment was higher than the eutrophication limiting target concentration of < 0.05 mg/L P; CC treatment demonstrated the potential this technology has for greatly reducing P export.

The brick-veg. and nonveg. treatments showed less consistent PRE. Initially, brick-veg. and nonveg. PRE were very high (99%), but after May, PRE in both declined slightly to around 89.7 ± 2.2 %, with outflow concentration averaging 0.5 ± 0.1 mg/L P (Fig. 4.6). Phosphorus removal efficiency for the brick-nonveg. treatment began to decline the second week of September and by October was exporting 1.6 ± 0.4 mg/L P, a concentration higher than the primary mesocosm's effluent. However, the brick-veg. treatment continued to effectively reduce P export to 0.4 ± 0.1 mg/L.

When SEC removal efficiencies were compared, CC-veg. treatments consistently fixed more P and maintained positive removal efficiency (80.2 ±0.02 %) in comparison with CC-nonveg (36.6 ± 0.06 %). Phosphorus removal efficiency by CC-nonveg. treatment was more variable and appeared dependent on inflow concentration from the primary treatment (Figs. 4.2 and 4.5). When primary treatment PRE was high and effluent concentration low, CC-nonveg. SEC removal efficiency declined, resulting in periods of P desorption. Conversely, when primary treatment PRE declined, CC-nonveg. SEC removal efficiency increased and P sorption to media resumed. Brick-veg. and nonveg. treatments exhibited similar trends with initially high PRE. Secondary PRE by the brick-veg. treatment was 67.7 ± 2.9 % and relatively consistent, similar to the CC-veg. treatment. However, brick-nonveg SEC PRE began to decline after May and by September consistent P desorption occurred. At this time, brick-nonveg. treatment P sorption sites were likely saturated and its substrate equilibrium P concentration was greater than the effluent concentration from the primary mesocosms.

From previous work we calculated that maximum P_{ad} sorption for brick was 0.5 g P / kg substrate (Chapter 2). This maximum P_{ad} indicated that binding sites should be saturated in the secondary mesocosms when 46.9 g of P has been fixed. Instead, sorption continued until 147.2 ± 4.5 g of P had been bound. However, the brick screened in our earlier sorption study was not the same brick that was used to fill the secondary treatment mesocosms. This brick substrate had a greater range of particle sizes. The resulting increased diversity of fine and coarse particles (and increased surface area available for P sorption), may account for much of the disparity between predicted P sorbed and actual P sorbed. Further, P migration from external to internal binding sites over time might have contributed to increased remediation efficiency of the brick used as a root-bed substrate compared (actual) with the initial 24-h sorption experiment (predicted). These results demonstrate that it is critical to monitor remediation efficiency of secondary treatment CWS. Basing expected substrate lifetime on sorption experiments alone does not account for change in sorption maximum over time or differences in P sorption capacity by brick source, however the sorption maximum determined from sorption experiments may yield a conservative estimate for substrate longevity.

The CC-nonveg. treatment fixed 1.8 g P / kg substrate and showed potential for additional P sorption because SEC PRE remained positive. A previous 9-month mesocosm study utilizing CC as a secondary treatment substrate found that the maximum quantity of P sorbed when CC was used as a secondary treatment ranged from 7.1 to 9.7

g P /kg substrate (Chapter 3). After comparison of the results of the previous study with the current one, it appears that the CC could potentially bind an additional 5.3 to 7.8 g of P / kg substrate, since PRE was still highly efficient and little detectable desorption had occurred.

The alkaline pH of the brick treatments may have increased the initial P sorption by the brick treatments. pH values were consistently > 9, which is basic enough to enhance formation of insoluble calcium-phosphate complexes that precipitate out of solution upon formation and may be deposited onto brick surfaces (Fig. S4.5). Calcium concentration in effluent of brick-veg and nonveg treatments were initially high, 401.2 ± 28.9 mg/L Ca^{2+} for the first sampling and 145.0 ± 56.0 mg/L Ca^{2+} for the second (Fig. S4.6). Thereafter, calcium concentrations among brick and CC treatments were similar. Grünberg and Kern (35) attributed high pH and Ca^{2+} concentrations in furnace slag with increased formation and precipitation of calcium phosphates. Brick-veg. treatment pH values were initially > 8.5, but, after the one month plant establishment period, effluent pH values decreased, eventually becoming similar to those of CC effluent. The presence of plant material in both CC and brick treatments resulted in a gradual pH decline, although the pH decline in the brick treatment was more dramatic.

Plant uptake of P accounted for the additional 105.9 ± 4.5 and 42.7 ± 3.9 g of P fixed by veg. brick and CC treatments, respectively (Table 4.1). The veg. secondary treatments had higher NRE and PRE than the nonveg treatments. The CC was more effective at polishing effluent water quality than the brick and showed potential for longer utility as a root-bed substrate. However, even though brick was not as efficient as

CC, it may be useful in settings where P remediation requirements are not rigorous, such as when effluent will be recycled for irrigation water. Further, since brick is a recycled product, it may be a more environmentally sustainable choice as a remediation substrate. These results indicate that a mixed primary surface-flow to secondary subsurface-flow CWS was able to maximize N and P removal from simulated nursery runoff. Primary surface-flow cells facilitated the majority of NRE, while secondary subsurface-flow cells facilitated the majority of phosphorus removal. Vegetated secondary treatment increased PRE and NRE, further reducing nutrients in effluent from the CWS.

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References

(1) National Agriculture Statistics Survey. Nursery crop 2006 summary. United States Department of Agriculture. Sp Cr 6-3(07). September 2007.

(2) Alexander, S.V. Pollution control and prevention at containerized nursery operations. *Wat. Sci. Tech.* 1993, *28*, 509-517.

(3) Arnold, M.A., Lesikar, B.J., Kenimer, A.L., Wilkerson, D.C. Spring recovery of constructed wetland plants affects nutrient removal from nursery runoff. *J. Env. Hort.* 1999, *17*(1), 5-10.

(4) Taylor, M.D., White, S.A., Chandler, S.L., Klaine, S.J., and Whitwell, T. Nutrient management of nursery runoff water using constructed wetland systems. *HortTech*. 2006, *16*(4), 610-614.

(5) Huett, D.O., Morris, S.G., Smith, G., Hunt, N. Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands. *Water Res*. 2005, *39*, 3259-3272.

(6) Lea-Cox, J.D., Ross, D.S., Teffeau, K.M. 2001. A water and nutrient management planning process for container nursery and greenhouse production systems in Maryland. *J. Env. Hort*. 2001, *19*, 230-236.

(7) U.S. Environmental Protection Agency. Definitions: Water quality standards criteria summaries: A compilation of state/federal criteria. U.S. EPA. Office of Water Regulations and Standards. U.S. EPA-440/5-88/017. 1988, Washington, D.C.

(8) Schipper, L.A., Vojvodic-Vukovic, M. Fiver years of nitrate removal, denitrification and carbon dynamics in a denitrification wall. *Wat. Res*. 2001, *35*(14), 3473-3477.

(9) Young, R.A., Huntrods, T., Anderson, W. Effectiveness of vegetated buffer strips in controlling pollution from feedlot runoff. *J. Env. Qual*. 1980, *9*(3), 483-487.

(10) Misra, A.K., Baker, J.L., Mickelson, S.K. Shang, H. Contributing area and concentration effects on herbicide removal by vegetative buffer strips. *Trans. ASAE*. 1996, *39*(6), 2105-2111.

(11) Lee, K.H., Isenhart, T.M., Schultz, R.C. Sediment and nutrient removal in an established multi-species riparian buffer. *J. Soil Wat. Conserv*. 2003, *58*(1), 1-8.

(12) Cooper, C.M., Moore, M.T., Bennett, E.R., Smith, S., Farris, J.L., Milam, C.D., Shields, F.D. Jr. Innovative uses of vegetated drainage ditches for reducing agricultural runoff. *Wat. Sci. Tech.* 2004, *49*(3), 117-123.

(13) Headley, T.R., Huett, D.O., Davison, L. The removal of nutrients from plant nursery irrigation runoff in subsurface horizontal-flow wetlands. *Water Sci. Tech.* 2001, *44*, 77-84.

(14) Akratos, C.S., Tsihrintzis, V.A. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. *Ecol. Eng*. 2007, 29, 173-191.

(15) Fraser, L.H., Carty, S.M., Steer, D., A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. *Bioresource Tech*. 2004, *94*(2), 185-192.

(16) Tanner, C.C. Plants for constructed wetland treatment systems – A comparison of the growth and nutrient uptake of eight emergent species. *Ecol. Eng*. 1996, *7*, 59-83.

(17) Gottschall, N., Boutin, C., Crolla, A., Kinsley, C., Champagne, P. The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. *Ecol. Eng*. 2007, *29*, 154-163.

(18) Kao, J.T., Titus, J.E., Zhu, W.X. Differential nitrogen and phosphorus retention by five wetland plant species. *Wetlands*. 2003, *23*(4), 979-987.

(19) Polomski, R.F., Taylor, M.D., Bielenberg, D.G., Bridges, W.C., Klaine, S.J., and Whitwell. T. Nutrient recovery by seven aquatic garden plants in a laboratory-scale subsurface constructed wetland. *HortSci*. 2007, in press.

(20) Engelhardt, K.A.M., Ritchie, M.E. Effects of macrophyte species richness on wetland ecosystem functioning and services. *Nature*. 2001, *411*, 687-689.

(21) Bachand, P.A.M., Horne, A.J. Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. *Ecol. Eng*. 2000, *14*, 17-32.

(22) Picard, C.R., Fraser, L.H., Steer, D. The interacting effects of temperature and plant community type on nutrient removal in wetland microcosms. *Biores. Tech*. 2005, *96*, 1039-1047.

(23) Kadlec, R.H., Reddy, K.R. Temperature effects in treatment wetlands. *Wat. Env. Res*. 2001, *73*(5), 543-557.

(24) Werker, A.G., Dougherty, J.M., McHenry, J.L., Van Loon, W.A. Treatment variability for wetland wastewater treatment design in cold climates. *Ecol. Eng*. 2002, *19*(1), 1-11.

(25) Kuschk, P., Wießner, A., Kappelmeyer, U., Weißbrodt, E. Kästner, M., Stottmeister, U. Annual cycle of nitrogen removal by a pilot-scale subsurface horizontal flow in a constructed wetland under moderate climate. *Wat. Res*. 2003, *37*, 4236-4242.

(26) Vymazal, J. Removal of nutrients in various types of constructed wetlands. *Sci. Tot. Env*. 2007, *380*(1-3), 48-65.

(27) Richardson, C.J. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science*. 1985, *228*(4706), 1424-1427.

(28) Pant, H.K., Reddy, K.R., Lemon E. Phosphorus retention capacity of root bed media of sub-surface flow constructed wetlands. *Ecol. Eng.* 2001, *17*, 345-355.

(29) Kadlec, R., Knight, R. *Treatment Wetlands.* CRC Press. 1996. Boca Raton, FL.

(30) Seo, D.C., Cho, J.S., Lee, H.J., Heo, J.S. Phosphorus retention capacity of filter media for estimating the longevity of constructed wetland. *Wat. Res.* 2005, *39*, 2445-2457.

(31) Prochaska, C.A., Zouboulis, A.I. Removal of phosphates by pilot vertical-flow constructed wetlands using a mixture of sand and dolomite as a substrate. *Ecol. Eng.* 2006, *26*, 293-303.

(32) Vohla, C., Alas, R., Nurk, K., Baatz, S., Mander, Ü. Dynamics of phosphorus, nitrogen and carbon removal in a horizontal subsurface flow constructed wetland. *Sci. Tot. En.* 2007, *380*, 66-74.

(33) Sistani, K.R., Mays, D.A., Taylor, R.W. Development of natural conditions in constructed wetlands: biological and chemical changes. *Ecol. Eng.* 1996, *12*, 125-131.

(34) Hume, H.P., Fleming, M.S., Horne, A.J. Plant carbohydrate limitation on nitrate reduction in wetland microcosms. *Wat. Res.* 2002, *36*, 577-584.

(35) Grüneberg, B., Kern, J. Phosphorus retention capacity of iron-ore and blast furnace slag in subsurface flow constructed wetlands. *Wat. Sci. Tech.* 2001, *44*(11-12), 69-75.

Tables

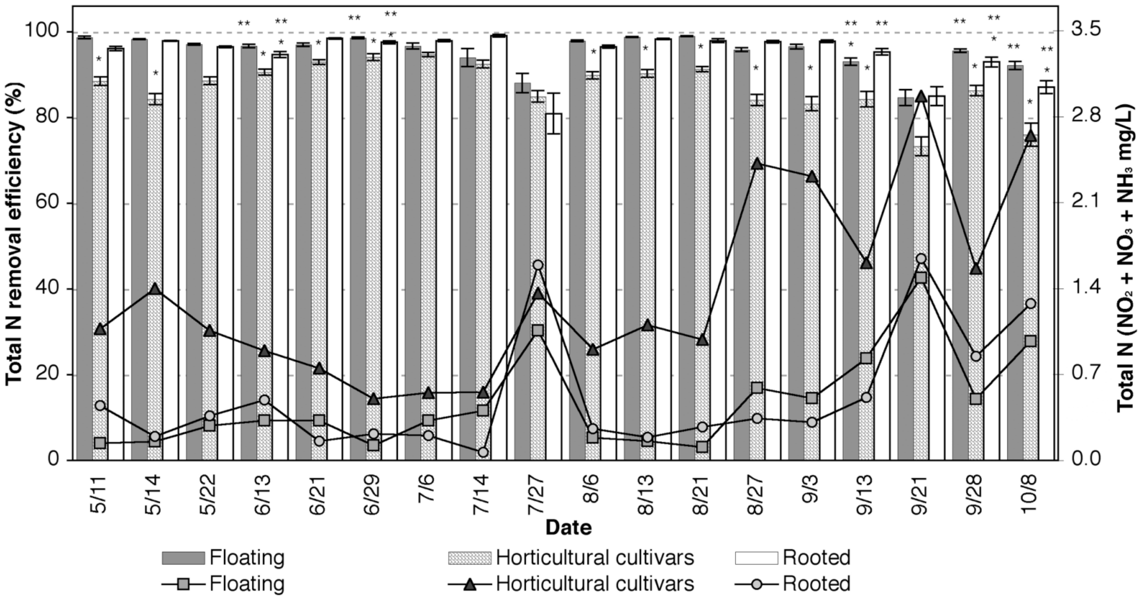
TABLE 4.1. Total nitrogen (TN) and total phosphorus (TP) fixation[a] in surface- to subsurface-flow mesocosms. Loading (in), export (out), and fixation (fixed), on a per mesocosm basis, were calculated based as the mean (standard error of the mean) for each treatment.

	TN In	TP In	TN OUT	TP OUT	[a] TN fixed system	TP fixed system	TN fixed secondary	TP fixed secondary
					(g)			
Initial	1966.2 (8.0)	911.0 (0.8)						
Primary treatments								
Floating			86.2 (1.9)	221.0 (3.2)	1913.2 (7.6)	690.4 (3.1)		
Rooted			95.4 (2.3)	195.3 (3.3)	1838.6 (7.1)	714.6 (3.3)		
Horticultural cultivars			250.7 (3.5)	349.8 (4.0)	1703.0 (7.3)	560.9 (4.0)		
Secondary treatments								
Brick: non-vegetated			183.5 (3.9)	103.5 (1.1)	1786.1 (9.8)	805.5 (1.6)	-5.3 (3.2)	147.2 (4.5)
Brick: vegetated			73.8 (1.7)	68.3 (1.0)	1892.5 (8.0)	844.2 (1.3)	113.8 (3.3)	253.1 (5.0)
Calcined clay: non-vegetated			186.4 (3.4)	44.3 (0.5)	1817.2 (9.1)	870.3 (1.1)	-100.1 (2.8)	166.9 (4.4)
Calcined clay: vegetated			84.0 (1.3)	24.1 (0.2)	1841.6 (7.1)	883.9 (0.7)	41.3 (3.1)	209.6 (3.4)

[a] System fixation calculated from ($T_{x, out} – T_{x, in}$); secondary fixation calculated from corresponding mesocosm ($Primary_{out}$ – $Secondary_{out}$).

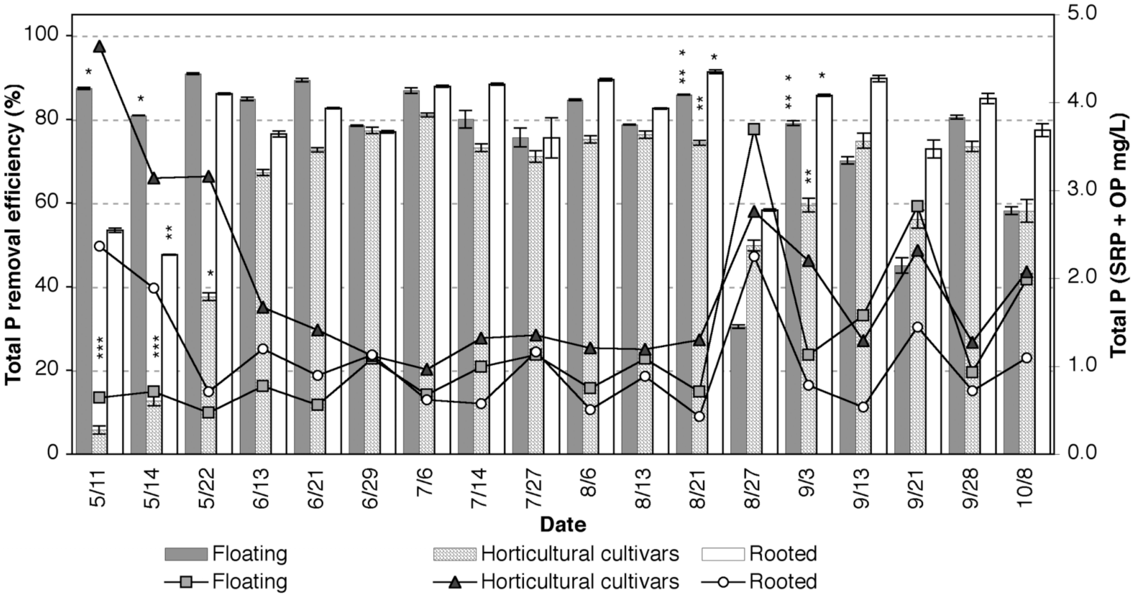
Figures

FIGURE 4.1. Total N removal efficiency (bars[a]) and effluent concentration (lines) of primary surface-flow mesocosms established with different planting types.



[a] Values are the average of 8 replicates per sampling period ± standard error of the mean. * represents statistically significant differences ($\alpha < 0.05$) at each sampling time.

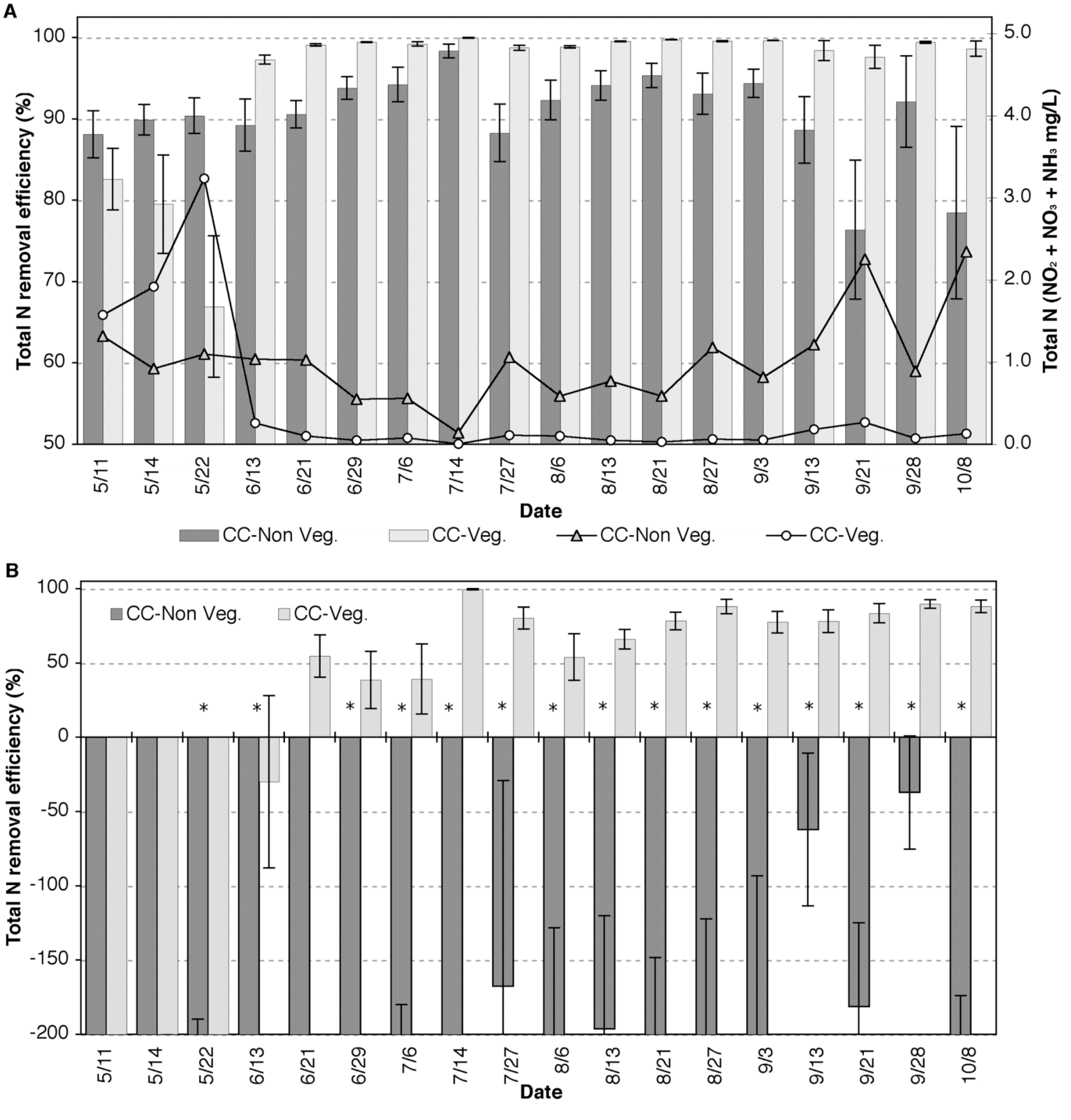
FIGURE 4.2. Total P removal efficiency (bars) and effluent concentration (lines) of primary surface-flow mesocosms established with different planting types.



[a] Values are the average of 8 replicates per sampling period ± standard error of the mean.

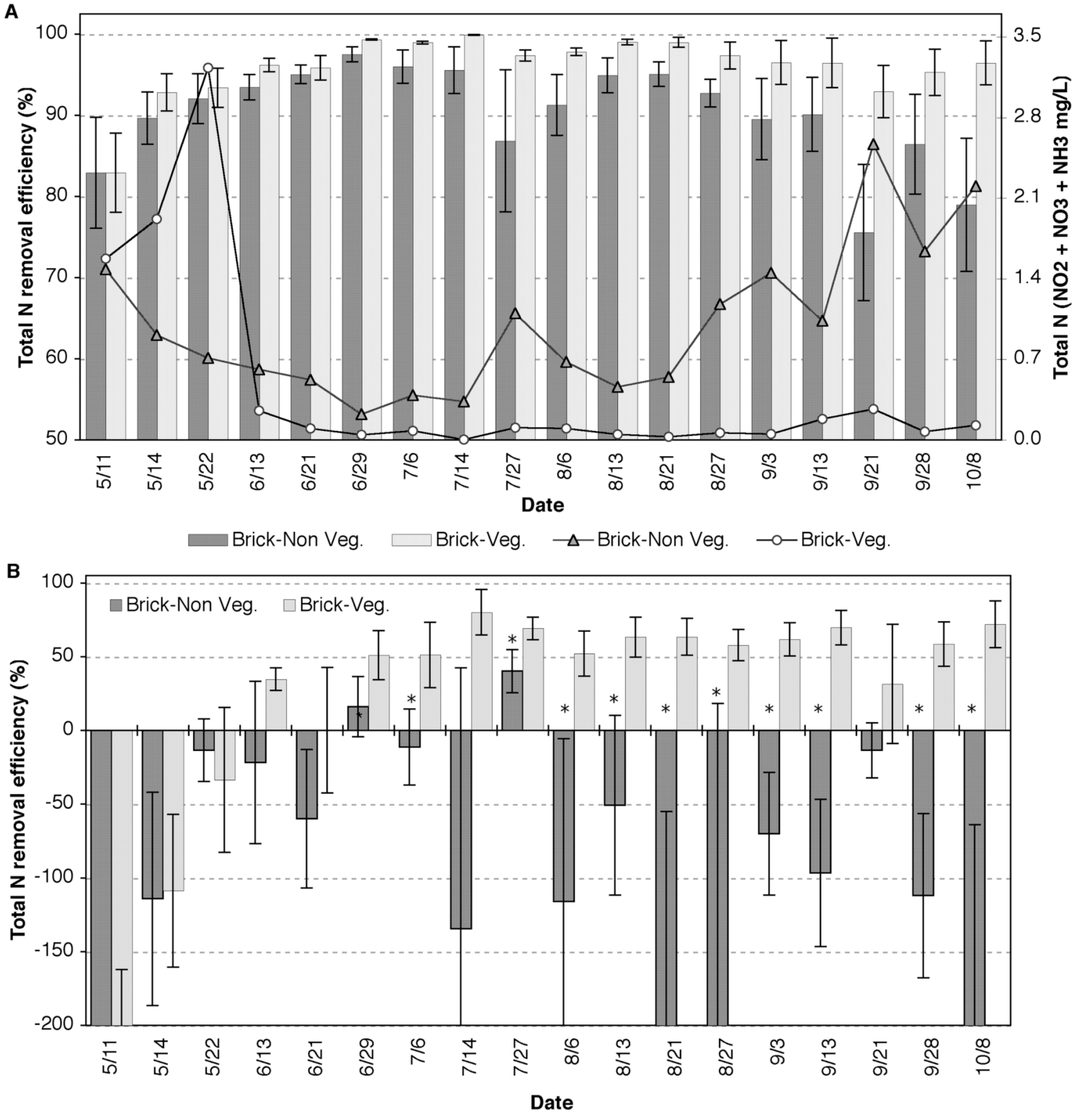
* represents statistically significant differences ($\alpha < 0.05$) at each sampling time.

FIGURE 4.3. Total N removal efficiency (bars) and effluent concentration (lines) of subsurface-flow mesocosms with calcined clay (CC) root-bed media. Mesocosms were either vegetated (Veg) or non-vegetated (Non Veg). (A) represents system [a] N removal efficiency and (B) represents secondary N removal efficiency.



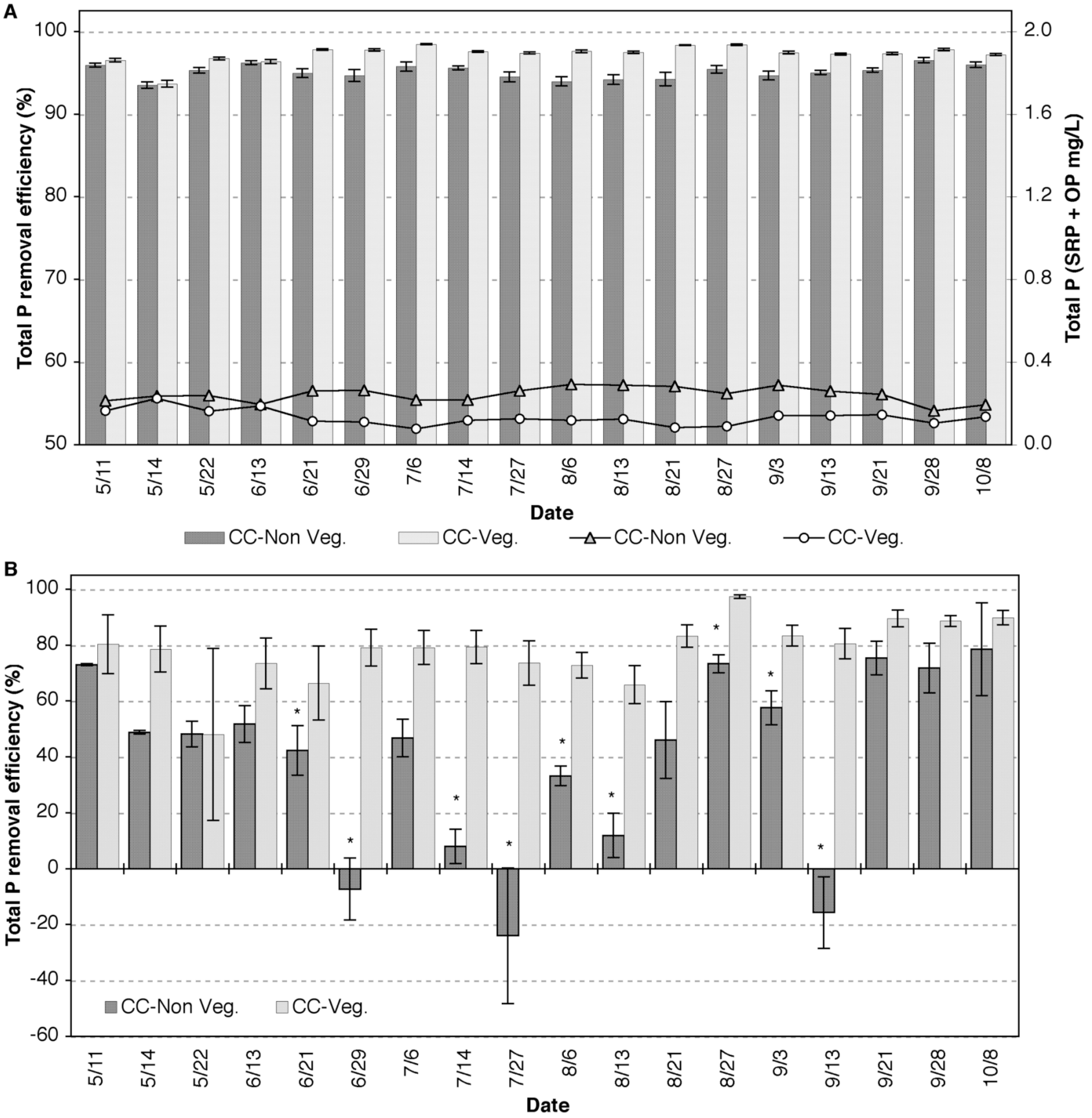
[a]System = [1 – ($N_{secondary}$ / N_{inflow}); Secondary = [1 – ($N_{secondary}$ / $N_{primary}$)]; * represent statistically significant differences ($P < 0.05$). Values are the average of 8 replicates ± standard error of the mean.

FIGURE 4.4. Total N removal efficiency (bars) and effluent concentration (lines) of secondary subsurface-flow mesocosms with brick root-bed media. Mesocosms were either vegetated (Veg) or non-vegetated (Non Veg). (A) represents system [a] N removal efficiency and (B) represents secondary N removal efficiency.



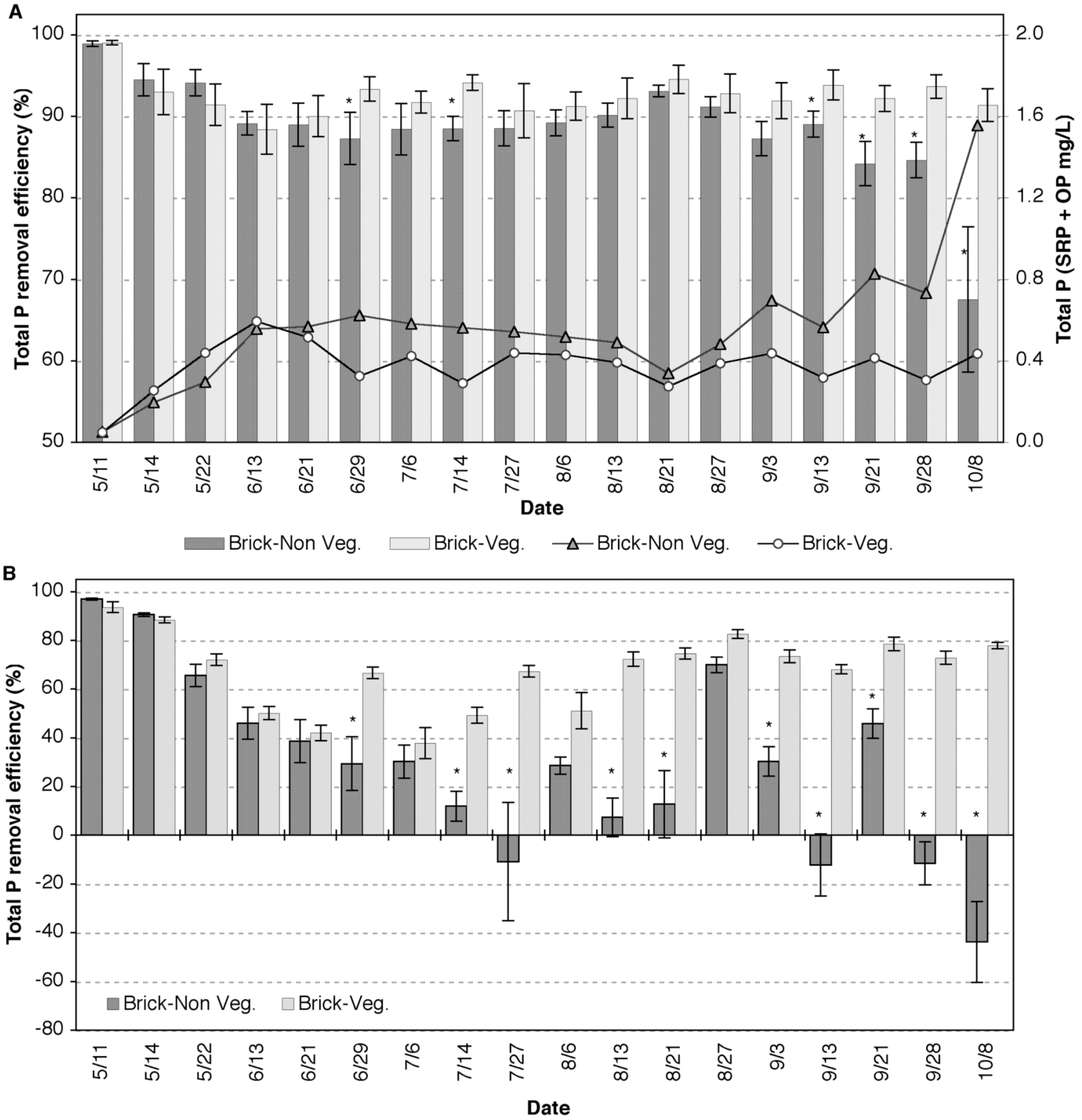
[a] System = [1 – ($N_{secondary}$ / N_{inflow}); Secondary = [1 – ($N_{secondary}$ / $N_{primary}$)]; * represent statistically significant differences ($P < 0.05$). Values are the average of 8 replicates ± standard error of the mean.

FIGURE 4.5. Total P removal efficiency (bars) and effluent concentration (lines) of secondary subsurface-flow mesocosms with calcined clay (CC) root-bed media. Mesocosms were either vegetated (Veg) or non-vegetated (Non Veg). (A) represents system [a] P removal efficiency and (B) secondary P removal efficiency.



[a]System = [1 – ($P_{secondary}$ / P_{inflow}); Secondary = [1 – ($P_{secondary}$ / $P_{primary}$)]; * represent statistically significant differences ($P < 0.05$). Values are the average of 8 replicates ± standard error of the mean.

FIGURE 4.6. Total P removal efficiency (bars) and effluent concentration (lines) of secondary subsurface-flow mesocosms with brick root-bed media. Mesocosms were either vegetated (Veg) or non-vegetated (Non Veg). (A) represents system [a] P removal efficiency and (B) secondary P removal efficiency.



[a] System = $[1 - (P_{secondary} / P_{inflow})$; Secondary = $[1 - (P_{secondary} / P_{primary})]$; * represent statistically significant differences ($P < 0.05$). Values are the average of 8 replicates ± standard error of the mean.

Supplementary Figures

FIGURE S4.1. Mesocosm primary surface-flow to secondary subsurface-flow CWS in May 2007 (A) and August 2007 (B).

FIGURE S4.2. Water-flow schematic from inflow tank through secondary treatment outflow.

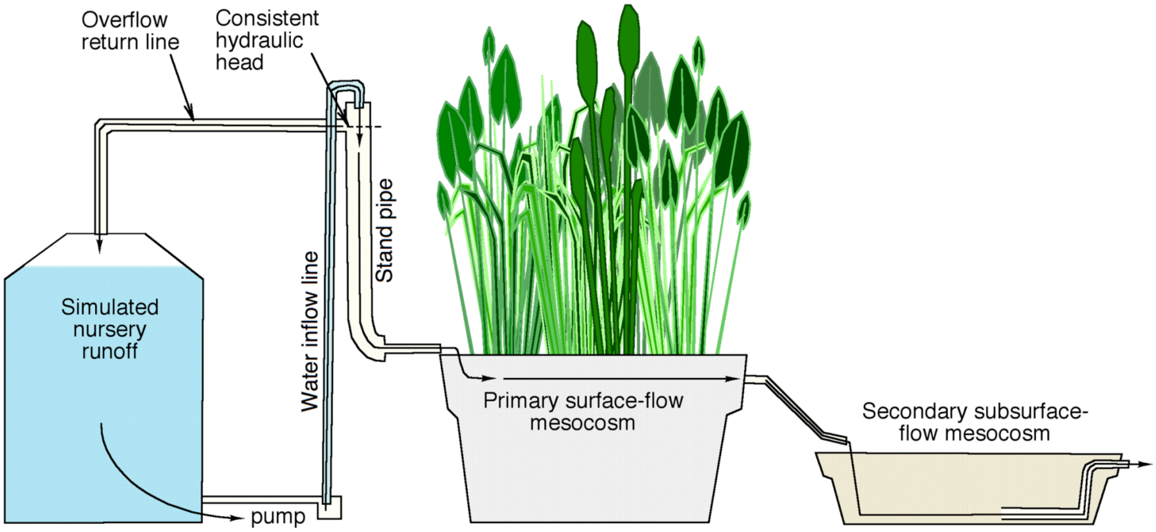


FIGURE S4.3. Average water temperature in primary mesocosms.

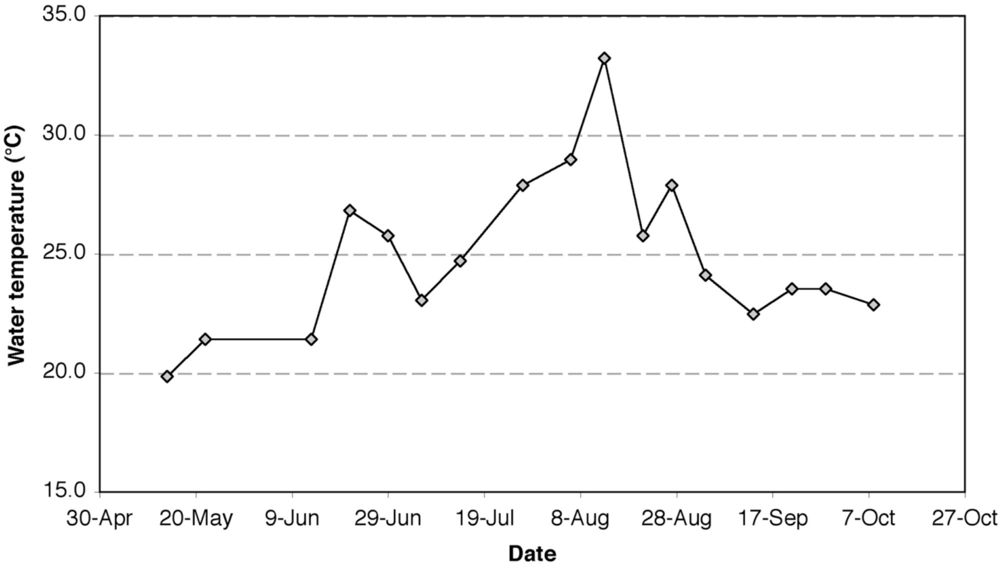


FIGURE S4.4. Average non-purgable organic carbon (NPOC) concentration by A) primary treatments (floating, rooted, and horticultural cultivars) and B) secondary treatments (brick vegetated and non-vegetated; calcined clay vegetated and non-vegetated). Data points are the treatment mean ± standard error of the mean.

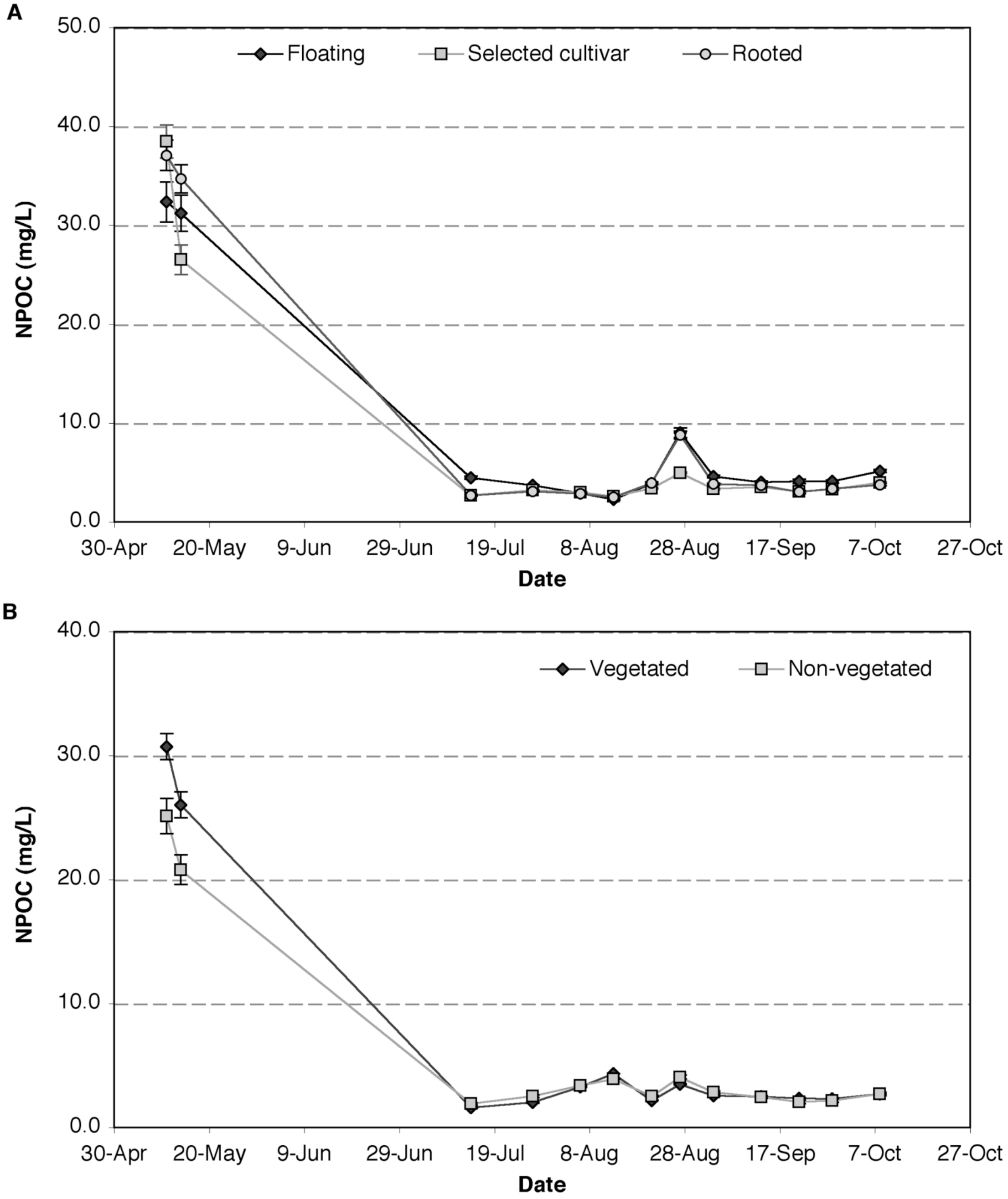


FIGURE S4.5. pH changes in primary (A, floating, rooted, and horticultural cultivar treatments) and secondary (B, brick vegetated (V) and non-vegetated (NV); CC, calcined clay vegetated and non-vegetated) mesocosm treatments.

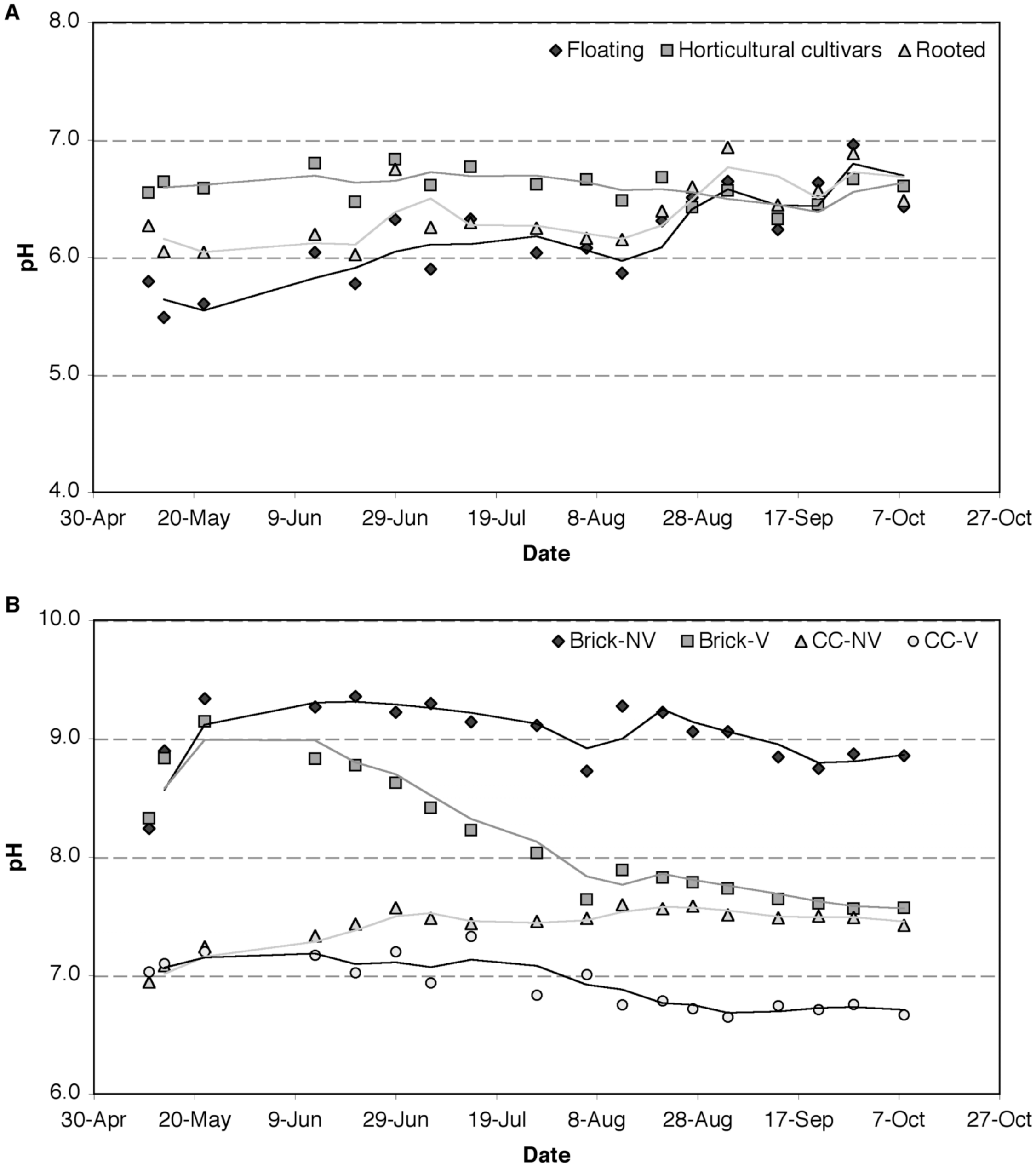
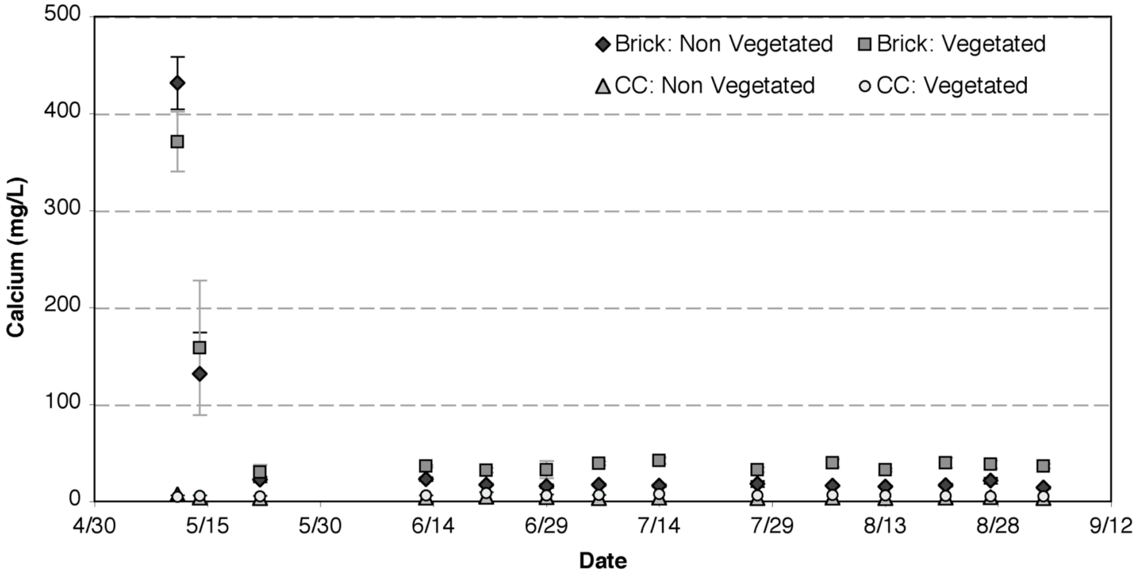


FIGURE S4.6. Calcium concentration in brick vegetated and non-vegetated, CC, calcined clay vegetated and non-vegetated secondary subsurface-flow treatments. Data points are the treatment mean ± standard error of the mean.



CONCLUSIONS

(1) A mixed surface- to subsurface-flow constructed wetland system (CWS) is more effective than either surface- or subsurface-flow CWS alone at nitrogen and phosphorus assimilation and removal.

(2) Nutrient loading rate must be known to design a CWS with adequate treatment area.

(3) A 4-day hydraulic retention time is adequate for nutrient remediation.

(4) The floating-mat planting style provided the greatest nitrogen removal efficiency.

(5) Horticulturally-significant cultivars did not exhibit optimum nutrient uptake at low nutrient loading concentrations.

(6) Plant biomass displaces 20-25% of water in CWSs.

(7) Brick and industrial mineral aggregate root bed substrates improve phosphorus remediation efficiency in secondary subsurface-flow CWSs.

(8) Industrial mineral aggregate polishes effluent water quality more effectively and sorbs phosphorus longer than brick.

(9) Planting secondary subsurface-flow CWSs further improves effluent water quality.

This research demonstrated that a mixed surface- to subsurface-flow constructed wetland system (CWS) was more effective at assimilating and removing nitrogen and phosphorus than either surface- or subsurface-flow CWSs alone. Nutrient loading rate must be known to design a CWS with an adequate treatment area. The high nutrient loading (> 30 mg/L N) treatment was not adequately managed by these CWS, but treatments with N loading < 18 mg/L were adequately remediated. The 4-day hydraulic retention time (HRT) treatment exhibited similar remediation efficiency to the 7-day HRT treatment. During winter 2005, the 7-day HRT treatment exported more P than the 4-day HRT treatment, regardless of nutrient load. Phosphorus concentrations in 4-day HRT treatment effluent were lower because P released from decaying plant material was more diluted by the faster flow rate of the 4-day HRT treatment than the 7-day HRT treatment.

Planting style is important when establishing CWSs. The floating-mat treatment provided the greatest N removal efficiency probably because the increased root surface area in the water column provided more attachment sites for microbial growth when compared with the rooted treatment. The horticulturally-important species examined were not as effective as native wetland species at nutrient removal. The decreased nutrient removal efficiency of horticultural-important species was a result of low (10 mg/L N) nutrient loading rates and the species' physiological predisposition to more efficient nutrient uptake at higher concentrations. Plant biomass displaced 22.9% of the water in the mesocosms. Thus, estimated CWS surface area should an increased by 20 to 25% to provide adequate treatment area and retention time.

Secondary subsurface-flow treatments exhibited consistent, highly efficient P removal until P binding site saturation occurred. After saturation, P desorbed from substrate, and effluent P concentrations were sometimes higher than inflow concentrations. Both calcined clay and brick reduced P concentrations in effluent. The calcined clay root-bed substrate more effectively polished effluent water than the brick and was able to sorb P from runoff for longer periods of time. However, dependent upon P removal efficiency needs, brick may reduce P concentrations in effluent to acceptable levels. Further, since brick is a recycled product, it may be a more environmentally sustainable product than calcined clay, which is mined and processed. Establishing plants in the subsurface-flow mesocosms further improved effluent water quality when compared with the non-vegetated secondary treatment, and both nitrogen and phosphorus concentrations were reduced. This mixed surface- to subsurface-flow CWS design shows great potential as a highly effective, low-maintenance treatment system for runoff from nursery operations.