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ORIGINAL ARTICLE

A Subsurface Upflow Wetland System for Removal of Nutrients and Pathogens in On-Site Sewage Treatment and Disposal Systems

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Abstract

On-site sewage treatment and disposal systems, commonly referred to as a septic systems, consist basically of a septic tank and soil adsorption field or drainfield. It may represent a large fraction of nutrient loads and pathogen impacts in vadose zone and groundwater systems. It includes not only nitrogen (N) and phosphorus (P), but also pathogen indicators such as fecal coliform and *Escherichia coli*, which indicate the presence of other disease-causing bacteria flowing into the aquatic system and potentially adversely affecting public health. Constructed wetlands, an effective small-scale wastewater treatment system with low energy and maintenance requirements and operational costs, will cover current needs for nutrient and pathogen removal. In our study, a next-generation subsurface upflow wetland system that is filled with green sorption media (e.g., mixes of recycled and natural materials) along with selected plant species was tested as a substitute for the conventional drainfield in septic tank systems. Four parallel subsurface upflow wetlands (i.e., three planted versus one unplanted) were built to handle 454 L/day (120 gallons/day) of septic wastewater flow. It proved effective in removing both nutrients and pathogens. During the test run in 2009, the planted wetlands achieved a removal efficiency of 84.2%, 97.3%, 98.93%, and 99.92% in total nitrogen, total phosphorus, fecal coli, and *E. coli*, respectively. A stress test conducted in winter 2010 successfully verified the reliability of this treatment process. Denitrification and precipitation were shown to be the dominant pathways for removing N and P, as evidenced by mass balance and real-time polymerase chain reaction analyses.

Key words: subsurface constructed wetland; green sorption media; biofiltration; wastewater treatment; nutrient and pathogen control; ecological engineering

Introduction

ALTHOUGH RESIDENTS IN towns and cities are generally served by centralized wastewater treatment facilities, >25 million homes, or 25% of the U.S. population, still use on-site wastewater treatment systems to meet their wastewater treatment and disposal needs (USEPA, 2003). Elevated nutrient and pathogen levels in groundwater may cause health problems in children (Walton, 1951) and may impair or destroy environmentally sensitive habitats (Beutel, 2006; Tarazona *et al.*, 2008). Increased nutrient and pathogen concentrations in surface waters may also lead to excess plant and algal growth and water pollution. When plants and algae die and decay, dissolved oxygen (DO) levels and overall water quality diminish.

The transfer of nitrogen from one phase to another is commonly referred to as the nitrogen cycle. When ammonia combines with organic materials, ammonium (NH_4^+) may be often created. In the presence of ammonia- and nitrite-oxidizing bacteria, ammonium is converted to nitrite (NO_2^-) and further to nitrate (NO_3^-) (Crites and Tchobanoglous, 1998). These two reactions are collectively called nitrification. Denitrification, conversely, performed by a denitrifying community, is an anaerobic respiration process using nitrate as a final electron acceptor. It results in stepwise microbiological reduction of nitrate, nitrite, nitric oxide (NO), nitrous oxide (N_2O) to nitrogen gas (N_2) (Crites and Tchobanoglous, 1998). Nitrate removal rates are directly influenced by the slow-growing bacteria that govern nitrification and denitrification. The optimal temperature for the denitrifiers was found to be between 10°C and 25°C (Sawyer *et al.*, 2003). Phosphorus removal is achieved through media sorption, chemical precipitation, plant uptake, and microbial assimilation (Farahbakhshazad *et al.*, 2000).

There are many ways for homeowners with septic tank systems to minimize the potential nutrient and pathogen impacts that on-site sewage treatment and disposal systems may have on the environment. The Florida Keys On-site Wastewater Nutrient Reduction Systems (OWNRS) Demonstration Project was initiated in 1995 to demonstrate the use of

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an OWNRS to reduce the concentrations of nutrients discharged to the coastal region of the Keys (Anderson *et al.*, 1998). One of the five treatment trains in the OWNRS was a septic tank followed by a recirculation sand filter. The overall passive on-site sewage treatment and disposal systems were shown to remove about 96.52% total suspended solid (TSS), 95.46% total Kjeldahl nitrogen, 47.58% total nitrogen (TN), and 92.84% total phosphorus (TP) (Anderson *et al.*, 1998). Septic tank effluent filters were evaluated based on site specificity (Byers *et al.*, 2001). A new combined distribution and pretreatment unit filled with lightweight clay aggregate for wastewater soil infiltration systems was applied (Heistad *et al.*, 2001). Healy *et al.* (2004) found removal efficiencies of 83.2% TN, 100% NH_4-N, 43.3% P, and 100% SS from dairy parlor washing, with 6.6 days hydraulic retention time (HRT) and a recirculation ratio of 3.0. Urynowicz *et al.* (2007) tried to evaluate the performance of recirculation sand filter in terms of nitrogen removal from septic tank wastewater and found 72.0% nitrogen removal with a recirculation ratio of 5.0 and 63.0% nitrogen removal with a recirculation ratio of 3.7.

To remove nitrogen (N) and phosphorus (P), a wide range of alternative on-site wastewater treatment systems were developed. Aerobic treatment units are designed to treat wastewater rather than the conventional method with septic tanks alone (Ivery, 1996). An aeration chamber is the most important compartment in aerobic treatment units: a pump supplying a constant flow of air and a stirring mechanism is used to oxygenate the water, creating optimum conditions for aerobic organisms to decompose organic compounds. The application of the aerobic treatment units may significantly reduce the health risk. The main disadvantages associated with aerobic treatment units are the need for an external power source and a higher maintenance level required to ensure proper system operation. A sand filter in conjunction with a septic tank or an aerobic treatment unit is an alternative that is commonly used to provide additional treatment for effluent. The main function of the sand filter is to reduce the amount of suspended solids and dissolved organic material present in the water. Microorganisms attached to the sand particles are able to aerobically digest the organic material within the wastewater. Havard *et al.* (2008) used six lateral flow filters (LFSFs) for the treatment of septic tank effluent. They evaluated the effects of slope and sand characteristics based on the satisfactory performance of LFSFs: biological oxygen demand (BOD) (98.5%), TSS (95.5%), and *Escherichia coli* (5.4 log reduction). Phosphorus removal ranged from 98% in the fine sand to 71.2% in the coarse sand filter. TN removal ranged from 60% to 66%. However, owners need to periodically rake and replace clogged surface sand. Regardless of the disadvantages of each of these two on-site wastewater treatment alternatives, it can be seen that denitrification in these two alternatives does not come up to expectations because of the presence of the aerobic environment. To date, the U.S. Environmental Protection Agency (USEPA) and numerous states are imposing stricter standards for the release of TN (as a combined measurement of ammonia-N, nitrite-N, and nitrate-N), phosphorus, and pathogenic bacteria by septic systems into conventional leach fields. Hence, there is an urgent need to find a more effective unit operation to help the septic tank system meet the upcoming USEPA regulations.

For some years, wetlands have been playing an important role in water conservation, climate regulation, soil erosion control, flood storage for use in drought, environment purification, etc. Based on the same principles for wastewater purification using natural wetlands, the man-made constructed wetland with effective management can enhance its ability to improve the effluent water quality. The wetland system removes nitrogen in the water through a variety of mechanisms including biological, physical, and chemical reactions. Its biological functions, such as ammonification, nitrification–denitrification and plant uptake under appropriate conditions, are regarded as crucial for nitrogen removal. Precipitation of a particular form of phosphorus is the main path for phosphorus removal. Microbial absorption and accumulation also play a role. Constructed wetlands can be divided into two main types: free water surface flow (SF) wetlands and subsurface flow (SSF) wetlands (EPA, 1993).

SF wetlands include emergent vegetation, soil or medium to support the emergent vegetation, and a water surface above the substrate. These kinds of constructed wetlands are particularly efficient in pathogen removal, because of the high exposure of wastewater to sunlight, but such a system provides an ideal terrain to breed mosquitoes and denitrification may be reduced because of exposure of the wastewater to air. In the SSF systems, the wastewater is fed in the inlet and passes the filter medium until it reaches the outlet zone. Because of the longer retention time of the wastewater in the filter, nitrogen reduction is significant with horizontal flow systems, but full nitrification is limited because of a lack of oxygen, which is characteristic for these kinds of systems. In the design philosophy of constructed wetland, how to optimally integrate the physical, chemical, and biological processes to remove nutrients by different kinds of sorption media and vegetation has attracted much attention throughout the world. Moreover, the potential of constructed wetlands for treating specific wastewater has been explored continuously as evidenced by a large body of literature. Steer *et al.* (2002) evaluated the effectiveness in improving water quality of a single-family septic tank/constructed wetland system in Ohio. They concluded that domestic treatment wetlands can reduce the output of fecal coliform to $88\% \pm 27\%$, TSS $56\% \pm 53\%$, BOD $70\% \pm 48\%$, ammonia $56\% \pm 31\%$, and phosphorus $80\% \pm 20\%$. Mbuligwe (2005) presented the performance of a coupled septic tank/engineered wetland system for treating and recycling from a small community. The coupled septic tank/engineered wetland system was able to remove ammonia by an average of 60%, nitrate by 71%, sulfate by 55%, chemical oxygen demand by 91%, and fecal coliform as well as total coliform by almost 100%. Tanaka *et al.* (2006) tested an integrated system of emergent plants and submerged plants to polish the effluent from a septic tank treating domestic sewage from a student dormitory. The overall pollutant removal efficiencies were 65.7% BOD, 40.8% chemical oxygen demand, 74.8% ammonium nitrogen (NH_4^+-N), 38.8% nitrate nitrogen (NO_3^--N), 61.2% phosphate (PO_4^{3-}), 65.8% TSS, and 94.8% fecal coliform.

A thorough review of the use of constructed wetlands with horizontal SSF for various types of wastewater in municipal, industrial, and agricultural sectors can be found in the literature (Vymazal, 2009). Various media have been studied and suggested. It was shown that a green sorption medium consisting of recycled and natural materials provides a favorable environment for nutrient removal (Xuan *et al.*, 2009).

Integration between different wetland species and green sorption media has not yet been examined to explore the best cost-effective and sustainable solution.

Thus, the objectives of this study were to assess (1) the performance of a subsurface upflow wetland (SUW) system for treating on-site wastewater; (2) the compliance to water quality standards by using innovative sorption media; and (3) the overall role of the planted vegetation. A full-scale pilot study was conducted to demonstrate the potential for application of such a passive on-site wastewater treatment system in central Florida.

Materials and Methods

Site description and experimental design

An SUW receiving septic effluent from BPW Scholarship House (a 15-person dormitory in the University of Central Florida) handles 454 m^3 (120 gallons) of influent per day in this wastewater treatment study. The septic tank before the SUW system has a size of 1,000 gallons/day, providing 2–3 days HRT. A gravel-filled gravity distribution system includes a header pipe, even distribution box, distribution pipe, flow meter, and four wetland cells packed with special green sorption media (recycled and natural materials). Within the full-scale field study, a new set of green sorption media was used for both nutrient and pathogen removal in the SUW. As gravel with higher porosity was used as the substrate at the bottom of each cell, an innovative upflow (i.e., outlet of SUW is higher than inlet) design was introduced to induce a uniform upflow hydraulic pattern and an amenable nitrification–denitrification environment as well as to avoid clogging and flooding, which overcomes the main disadvantage of conventional SSF wetlands. Besides, such a design might result in maximal reduction of the effect of stormwater. In a conventional SSF wetland (i.e., inlet is higher than outlet), the stormwater moves downward, permeates the whole cell, and finally drains out of the bottom of the cell with wastewater in rainy days. For our SUW, the water level in the cell was kept as high as the outlet, and part of stormwater tended to drain from the higher outlet directly instead of mixing with the wastewater beneath. So less stormwater would reach the sampling ports, which allowed us to make a more accurate evaluation of our designed media performance. Through various physical, chemical, and biological processes, most bacteria and viruses in wastewater, as well as nutrients, were consumed and intercepted as the wastewater effluent travels up through the sand layer (i.e., aerobic layer at the bottom) and pollution control layer (i.e., anaerobic layer in the middle) until the growth media (G media) layer. Combined with the gravel layer placed beneath the sand layer and the plant species inserted in the G media, the unique SUW might promote the pathogen, nitrogen, and phosphorus removal via nitrification, denitrification, adsorption, absorption, ion exchange, filtration, and precipitation collectively. Three kinds of plant species were individually planted in three cells against the parallel control case with no plant species (Fig. 1). The DIGI-FLOW™ F-1000RT paddlewheel meter is a battery-powered digital flow meter for full-pipe water flow measurement. It features a total and an instantaneous flow measurement. Such an individual paddlewheel flow meter was installed at every entrance and exit of the four wetland cells to record the inflow and the outflow and further to determine the mass balance at the wetland system. A totalizer was used to measure the daily total flow of the wetlands.

There were four parallel 1.52 m wide×3.05 m long×0.91 m deep (5 feet wide×10 feet long×3 feet deep) cells in this test bed. As shown in Fig. 2, each of the four cells contained an impermeable liner, a gravel substrate, fabric interlayer, sand, pollution control media (PC media), G media, and selected plants. The main function of the G media layer (75% expanded clay, 10% vermiculite, and 15% peat moss) was to support the root zone and to speed up the maturation process of the treatment system. The 30.48-cm (12-inch) PC medium layer (50% citrus grove sand, 15% tire crumbs, 15% sawdust, and 20% lime stone) was used to help nutrient, TSS, and BOD removal. A 15.24-cm (6-inch) sand layer was then added beneath the PC medium to improve the removal of pathogen and TSS. The 30.48-cm (12-inch)-thick gravel substrate created additional pore space, allowing water to spread across the bottom of wetland more freely while maintaining a certain flow rate. The purpose of the separation fabric liner on top of the gravel layer was to keep the sand stable above the gravel layer. Once the gravel layer was fully saturated, the water level would rise up gradually, passing through the sand and PC medium layer up to the outlet. Chowdhury *et al.* (2008) gave an overview of flow pattern in an SUW by conducting a bromide tracer study. They found that, in a bottom inlet and top outlet condition, a gravel layer added at bottom caused the flow to be mostly in the vertical direction, which provided strong evidence for our hydraulic pattern hypothesis. In each

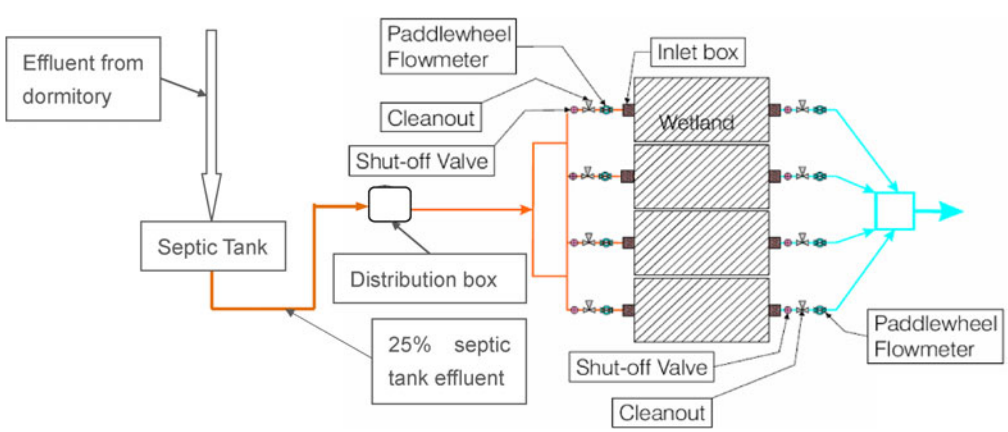

FIG. 1. Configuration of a septic tank followed by four-cell wetlands for treating 25% of the septic effluent. For comparison, the remaining 75% was sent to other wastewater treatment facilities in the same pilot plant.

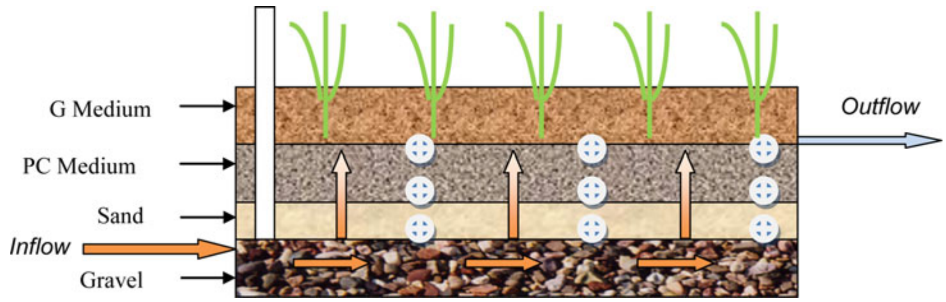

FIG. 2. Sectional view of wetlands. G medium, growth medium; PC medium, pollution control medium.

wetland, two customized oxygenators (polyvinyl chloride pipe wrapped with fabric at bottom) were inserted on both sides of inlet into the gravel layer to enhance nitrification at the bottom of the wetland cells so as to fulfill the design ideas configured for the SUW. The samplers (airstone connected with rubber tubing to the surface) were installed at the interface between different layers with three depths. Horizontally, the samplers in the four wetland cells are 33%, 67%, and 100% along the length of the wetland (Fig. 2). Samples identified here were defined for the discussion as follows: (1) "port B": mixture of bottom three samples, (2) "port M": mixture of middle three samples, (3) "port T1": top sample at 1/3 length, (4) "port T2": top sample at 2/3 length, and (5) "port T3": top sample at 3/3 length.

A tracer study can directly determine the direction and velocity of water movement by monitoring the matter or energy carried by water and can indirectly investigate some other hydrologic parameters such as dispersivity, porosity, hydraulic conductivity, etc., through further analysis. An ideal tracer should be representative, which means it follows the same path as the water and requires the following main characters: easy detection, inexpensive analysis procedure, low toxicity, high solubility, and low background nature. There are three most popular choices for a tracer: isotope, ions, and dyes that have been used in many applications (Lin *et al.*, 2003; Kadlec *et al.*, 2005; Małoszewski *et al.*, 2006; Ronkanen and Kløve, 2007, 2008; Giraldi *et al.*, 2009). Because of the advantages of low detection limits, zero natural background, low relative cost, and easy on-site analysis, in our study, Rhodamine WT was selected as the water tracing dye to determine the HRT of the wetland cells.

Selection of plant species

As components of wetland system, plant species have an irreplaceable function in pollutant purification. In the subsurface wetland system, the biofilm around the plant rhizosphere provides a potential attachment site for bacteria to achieve the nitrification and the aerobic degradation of soluble organics. Based on the characteristics of oxygen transmission, it showed an aerobic–anoxic–anaerobic state around the rhizosphere, the equivalent of a series of parallel anaerobic–anoxic–oxic (A^2O) processing units, which is a commonly used nutrient removal process in wastewater treatment. Aerobic areas near the root zone were conducive to nitrification and anaerobic areas away from the roots work for denitrification, both of which might perform the final clean-up of residual nitrogen in the septic effluent. Nitrate would thus be effectively removed by denitrification in rhizospheric zones. TN and TP can be removed if the plants are harvested routinely. Seidel's work (1955) is known as the first attempt to use the wetland vegetation to remove various pollutants from wastewater. Since then, researchers have untiringly planted different vegetations in an attempt to arrive at more desirable pollutant removal efficiencies. Table 1 reviews literature in which different kinds of vegetation with natural soil were used as substrates for wastewater treatment.

From Table 1, only *Phragmites australis* (in case 1b and 1f in SF) showed a good result with respect to the removal of nutrients (about 90% TN removal). However, *P. australis* is a kind of typical emergent vegetation, which is unsuitable for planting in our SUW. Under the criteria for screening plant species described in the previous study (Xuan *et al.*, 2009), three kinds of native vegetation with the same volume and net price, canna (*Canna flaccida*), blue flag (*Iris versicolor* L.), and bulrush (*Juncus effusus* L.) (Fig. 3), were ultimately selected and evenly planted in wetland cells 1, 2, and 3, respectively. Seedlings of these three kinds of plant were purchased from a local nursery and planted 2 months before the experiment period (7–8 plants/m^2). Wetland cell 4 is the control case without any plant species except the placement of the same layered green sorption media.

Selection of green sorption media

The importance of developing specific wetland porous media instead of conventional soil, sand, and gravel to gain better pollutant removal capacity has been widely recognized. Mann and Bavor's work (1993) represented the pioneer trial in the early period, in which the comparison of laboratory-scale phosphorus adsorption was conducted between regional gravels and alternative adsorptive media including industrial slag and ash byproducts. They reported a maximum adsorption capacity of regional gravels of 25.8–47.5 μg P/g, blast furnace slag 160–420 μg P/g, and fly ash 260 μg P/g, which warranted further research involving the inclusion of industrial waste substrata. Coombes and Collett (1995) used crushed basalt and limestone chippings in their horizontal flow *P. australis* wetland. Ammoniacal nitrogen in effluent had averaged <2 mg/L. Pant *et al.* (2001) found that the Fonthill sand had a better performance in removing P from wastewater in a comparison between three types of media (Lockport dolomite, Queenston shale, and Fonthill sand). Vohla *et al.* (2007) tried a designed oil-shale ash derived from oil-shale combustion for P retention. The life cycle time was not 8 years working as calculated from laboratory batch experiments, but several months because of the possible saturation or clogging in terms of quick biofilm development on the ash particles. Korkusuz *et al.* (2007) carried out an investigation of blast furnace granulated slag. They showed that blast furnace

Table 1. Wetland Performance Based on Different Kinds of Vegetation

SF	*Plant*	*Removal efficiency*	*Reference*
1a	*Typha latifolia*, *Phragmites australis*, *Sparganium erectum*	80% COD, 83% BOD, 45% TN, 47% TP	Cadelli *et al.* (1998)
1b	*P. australis*	98% SS, 87% COD, 96% BOD, 91% TN, 60% OrthoP	Cadelli *et al.* (1998)
1c	*P. australis*, *Scirpus lacustris*	68% COD, 83% BOD, 26% TN, 2% OrthoP	Cadelli *et al.* (1998)
1d	*Lemna* sp.	96% SS, 75% COD, 90% BOD, 43% TN, 47% TP	Cadelli *et al.* (1998)
1e	*Lemna* sp.	98% SS, 96% COD, 94% BOD, 49% TN, 49% TP	Cadelli *et al.* (1998)
1f	*P. australis*	87% COD, 97% BOD, 89% TN, 46% TP	Cadelli *et al.* (1998)
SSF	*Plant*	*Removal efficiency*	*Reference*
1	*Phragmites*	90% COD, 96% BOD, 92% SS, 63% TP, 36% TN	Haberl *et al.* (1998)
2	*Scirpus cyperinus*, *T. latifolia*	73.4% NH_4^+-N, 67.5% TKN	Huang *et al.* (2000)
3a	*T. latifolia*, *Typha angustofolia*, *Scirpus taebormontanii*	92% BOD, 87% TSS, 99.6% fecal, 41% TN, 50% TP	Henneck *et al.* (2001)
3b	*Typha* sp.	82% BOD, 86% TSS, 92.4% fecal, 51% TN, 59% TP	Henneck *et al.* (2001)
3c	*T. latifolia*	83% BOD, 81% TSS, 99.9% fecal, 54% TN, 97% TP	Henneck *et al.* (2001)
4a	*Phragmites mau*, *Ritianus*	25.2% NH_4^+-N, 56.3% COD, 57% TC, 68% FC	Kaseva (2004)
4b	*T. latifolia*	23% NO_2-N, 23% NH_4^+-N, 60.7% COD, 60% TC, 72% FC	Kaseva (2004)
5a	*Cyperus papyrus*	75.3% NH_4^+-N, 83.2% TRP	Kyambadde *et al.* (2004)
5b	*Miscanthidium violaceum*	61.5% NH_4^+-N, 48.4% TRP	Kyambadde *et al.* (2004)
6	*P. australis*	30% of TP, 50% denitrification	Brix and Arias (2005)
7	*Phragmites* and *Typha*	27% TKN, 19% NH_4^+-N, 4% nitrite	Keffala and Ghrabi (2005)
8a	*Juncus effusus* L.	54% NH_4^+-N, 55% TN, 95% TP	Xuan *et al.* (2009)
8b	*Panicum hemitomon*	88% NH_4^+-N, 85% TN, 94% TP	Xuan *et al.* (2009)
8c	*Zizaniopsis miliacea*	78% NH_4^+-N, 79% TN, 95% TP	Xuan *et al.* (2009)

BOD, biochemical oxygen demand; COD, chemical oxygen demand; FC, fecal coliform; NH_4^+, ammonium; NH_4^+-N, ammonium-nitrogen; NO_2^-, nitrite; NO_2-N, nitrite-nitrogen; SF, surface flow; SSF, subsurface flow; TC, total carbon; TKN, total Kjeldahl nitrogen; TN, total nitrogen; TP, total phosphorus; TRP, total reactive phosphorus; TSS, total suspended solid.

granulated slag have high phosphorus (P) sorption capacity, removing TP concentrations from 6.61 ± 1.78 to $3.18 \pm 1.82\,mg\,L^{-1}$ because of its higher Ca content and porous structure. Park and Polprasert (2008) investigated the ability for P removal using an integrated constructed wetland system packed with oyster shells as adsorption and filtration media. The removal efficiency of the integrated system was found to be 85.7% of N and 98.3% of P. Tee *et al.* (2009) reported a better performance of planted constructed wetlands with gravel and raw rice husk-based media for phenol and nitrogen removal compared with unplanted ones. Against such a background, the study generated a suite of six criteria for screening competitive sustainable sorption media: (1) the relevance of the nitrification or denitrification process or both, (2) the hydraulic permeability, (3) the cost level, (4) the removal efficiency as evidenced in the literature with respect to adsorption, precipitation, and filtration capacity, (5) the availability of media in Florida, and (6) additional environmental benefits. The differences of the new sorption media to the other types of media studied previously can be seen in the literature (Ryan *et al.*, 2009). After a long period of trial and error, the final recipe adopted in this study was (1) PC media: 50% citrus grove sand, 15% tire crumbs, 15% sawdust, and 20% lime stone; and (2) G media: 75% expanded clay, 10% vermiculite, and 15% peat moss. All of the percentages are by volume (Xuan *et al.*, 2010).

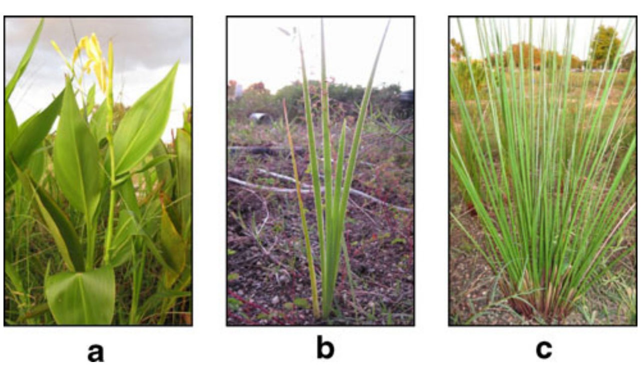

FIG. 3. Plant species selected in the study: **(a)** canna; **(b)** blue flag; **(c)** bulrush.

Sampling and analysis

A 24-h composite sample (a representative sample combined by multiple samples at regular intervals) was taken at every sampling port in proportion to the actual flow load during 24 h. Fecal coliform and *E. coli* samples were collected in a 100-mL sterile polyethylene flask. The container was immediately sealed, labeled, and measured by the external certified laboratory within the same day. A clean polyethylene jug was used to store each sample for analysis of other parameters. Once the samples were taken, the containers were stored in a chilled cooler (4°C) until the 24-h composite samples were completed. Samples that required appropriate preservatives were processed according to a quality assurance/quality control protocol. Two hundred milliliters of each sample was filtered through a 0.45-μm filter. One hundred milliliters of the filtered samples was set at pH <2.0. Each sample was delivered to the external certified laboratory in an appropriate ice chest within the same day to ensure the integrity of the samples.

A mass balance analysis was conducted based on the measurements during a 1-month study period. The TN in media was measured as the amount being sorbed in media during the wastewater treatment process. N_2 discharge into the air was calculated as the residual term in the TN budget. In the same way, the TN and TP in influent and effluent were calculated as the product of water flux and nutrient concentration. Porosity in different layers may be taken into account to finally summarize the amount of TN and TP in pore water. The amount of phosphorus released from the plants can be ignored, provided no fading leaves fell in the given period. The amount of uptake and release by microbes can be balanced out because there is a dynamic equilibrium between uptake and release by microbes. Because of technical complexity, precipitation in septic wetland was calculated as the residual term of the phosphorus budget. Ultimately, the nitrogen and phosphorus budget can be simplified by the following equations:

$$\begin{aligned}\text{In} = {} & \text{Out} + \text{Uptake by plant} + \text{Sorption in media} \\ & + \text{Storage in wastewater} + N_2 \text{ discharge by} \\ & \text{denitrification} \end{aligned} \tag{1}$$

$$\begin{aligned}\text{In} = {} & \text{Out} + \text{Uptake by plant} + \text{Sorption in media} \\ & + \text{Storage in wastewater} + \text{Precipitation} \end{aligned} \tag{2}$$

At the beginning and the end of the study period, samples of PC media and G media were randomly collected from each wetland cell and mixed together to form a composite sample filled in a 1-L (quart size) resealable plastic bag. Similarly, samples of leaf and root were collected in a random $30\times30\,cm^2$ area and analyzed for measuring TN and TP uptake by plant tissue.

The water quality in the wetland system was monitored weekly from September 2 to September 30 in 2009. DO, pH, and temperature were measured on-site by HACH HQd field case. In addition to those parameters mentioned above requiring a grab sample analysis, ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), organic nitrogen-nitrogen (ON-N), TN, organic phosphorus-phosphorus (OP-P), soluble reactive phosphorus-phosphorus (SRP-P), and TP were measured by a certified lab (Table 2). All media and plant samples were collected on September 2 and September 30 as representative samples of the initial and final stages of the experimental period. Media samples were delivered to a soil laboratory in the Pennsylvania State University for analysis. Plant samples were sent to an agricultural lab located in Orange City, Florida. For real-time polymerase chain reaction (PCR) analysis at the University of Central Florida, each media sample from different wetlands was collected into a 1.5-mL microcentrifuge tube and kept at $-20°C$ until use.

The detailed procedure of deoxyribonucleic acid (DNA) extraction and the real-time PCR was well addressed in a companion study (Xuan *et al.*, 2009). The real-time PCR was applied to gain insight into the denitrifiers' activity across the green sorption media given that the substrate and enzyme are not the limiting factors in the treated effluent. Both PC and G medium samples from each wetland were collected into 1.5-mL microcentrifuge tubes and kept at $-20°C$ until use. DNA from the sample was extracted in duplicate by using a SoilMaster® DNA Extraction Kit (EPICENTRE) and 50 mg instead of 100 mg of the sample (default value in manufacturer's instructions) was weighed into the 1.5-mL microcentrifuge tube to decrease the effects of enzymatic inhibitors. Three hundred microliters of extracted DNA template was finally prepared. Real-time PCR quantification was performed on the Stepone® (Applied Biosystems) PCR instrument to amplify *nirK* gene from the denitrifiers. A pair of primers, nirK876 (5′-ATYGGCGGVCAYGGCGA-3′) and nirK1040 (5′-GCCTC GATCAGRTTRTGGTT-3′) (Braker *et al.*, 1998), were used to amplify the target gene. The PCR mixture was prepared in a total volume of 25 μL using 12.5 μL of the SYBR Green PCR Master Mix kit, 10 μM of each primer, standard DNA or extracted DNA from samples, and diethylpyrocarbonate-treated water to complete the 25 μL volume. The PCR protocol for *nirK* real-time PCR was 120 s at 50°C, 900 s at 95°C, and then, six touchdown cycles: 15 s at 95°C for denaturation, 30 s at 63°C for annealing, 30 s at 72°C for extension, and 30 s at 80°C for a final data acquisition step. The annealing temperature was progressively decreased by 1°C down to 58°C. Finally, a last cycle with an annealing temperature of 58°C was repeated 40 times (Henry *et al.*, 2004).

Table 2. Outline of Analysis Methods

Parameter	*Analytical method*	*Testing location*
pH	USEPA 150.1	On-site
Ammonia-N	EPA 350.1	Certified lab
Nitrite-N	EPA 353.2	Certified lab
Nitrate-N	EPA 353.2	Certified lab
Organic nitrogen-N	EPA 350.2	Certified lab
Soluble reactive phosphorus-P	EPA 365.3	Certified lab
TP	Alkaline persulfate digestion	Certified lab
Dissolved oxygen	Manufacturer manual	On-site
Temperature	Manufacturer manual	On-site
Uptake by plant	N: Kjeldahl digestion P: wet digestion with nitric and perchloric acids	Agricultural lab
Nutrient sorption in media	DTPA saturated media extract method	PSU lab
Quantity of denitrifiers	Real-time PCR	UCF lab

DTPA, diethylene triamine pentaacetic acid; PCR, polymerase chain reaction; PSU, Pennsylvania State University; UCF, University of Central Florida; USEPA, U.S. Environmental Protection Agency.

Table 3. Water Quality Variation in Different Cells of Subsurface Upflow Wetland (Mean ± Standard Deviation)

Parameter	*After septic*	*Wetland 1*	*Wetland 2*	*Wetland 3*	*Wetland 4*
FC (10^3cfu/100 mL)	905.8 ± 760.9	0.071 ± 0.111	1.738 ± 1.493	0.077 ± 0.122	0.081 ± 0.132
Escherichia coli (10^3 cfu/100 mL)	480.0 ± 527.8	<0.001	0.017 ± 0.028	0.063 ± 0.099	0.003 ± 0.002
BOD_5 (mg/L)	44.5 ± 22.7	4.4 ± 2.1	4.3 ± 2.4	5.7 ± 1.9	9.6 ± 4.5
$CBOD_5$ (mg/L)	28.6 ± 11.7	3.2 ± 0.9	3.5 ± 1.4	4.7 ± 1.5	5.2 ± 0.7
NH_3-N (mg/L)	56.1 ± 2.2	0.8 ± 0.5	1.2 ± 0.2	21.5 ± 15.7	51.8 ± 23.1
TN (mg/L)	69.7 ± 4.8	2.1 ± 0.5	4.8 ± 4.2	25.7 ± 18.7	62.8 ± 19.2
SRP (μg/L)	3,974.5 ± 557.3	18.3 ± 7.5	39.0 ± 44.0	14.5 ± 6.5	27.3 ± 31.3
TP (mg/L)	5,574.3 ± 180.5	96.0 ± 35.2	242.0 ± 227.4	111.5 ± 36.8	799.5 ± 402.6

CBOD, carbonaceous biochemical oxygen demand; SRP, soluble reactive phosphorus.

Results and Discussion

Pathogen and nutrient removal

According to the tracer study, each of the wetland cell has ~3 days of HRT based on the influent of 0.11 m^3/day (30 gallons/day) on average. The wetland system was monitored weekly from September 2 to September 30, four times in total (Tables 3 and 4). During the four runs, the mean count of fecal coliform in influent was 907,000 cfu/100 mL. The mean influent count of *E. coli* was 480,000 cfu/100 mL. These wetlands individually reduce fecal coliform from 99.13% to 99.98% and *E. coli* from 99.80% to 100% (Table 4). Pathogen counts in effluent from each cell were below the EPA recommended value of 1,000 counts/100 mL in 93.8% of the samples (30 of 32). Half of the effluent samples were below the EPA maximum contaminant level standard, which requires 0 cfu of both fecal coliform and *E. coli* for drinking water. Wetland 1 always achieved 0 cfu of *E. coli* and 100% removal efficiency and showed a different pattern for fecal coliform (Fig. 4a). Port M had the best results and port T3 performed better than ports T1 and T2. In the rest of the cells, the highest removal efficiency appeared in port T1 and gradually increased until port T3. A short cut near the outlet could cause this result.

Figure 5 shows the DO in different cells. The record confirms that the oxygenators installed had, as expected, promoted the DO level to some extent. The average DO increased sharply from 1.25 mg/L in septic effluent to 3.26 in port B and then slightly decreased layer by layer. The extremely high DO value and variation in port T3 of cell 2 was caused by excessive siphoned air when the wastewater was insufficient to be collected. For nutrient removal, Fig. 6 illustrates the variation of NH_3-N and TN concentrations in the septic tank effluent and effluent from the four constructed wetland cells during four runs. The mean concentration of ammonia in the septic effluent was 56.1 ± 1.9 mg/L in the septic tank effluent. Mean ammonia removal efficiencies of the wetland system were 98.6%, 97.9%, 60.2%, and 8.1% in wetland cells 1, 2, 3, and 4, respectively. TN concentration of wastewater after septic tank (wetland influent) ranged from 64.8 mg/L (September 23) to 75.4 mg/L (September 9, 2009). The mean concentration of TN in the septic tank effluent was 69.7 ± 4.1 mg/L. The mean concentrations of TN in the effluent from four wetlands were 2.1 ± 0.4, 4.8 ± 3.7, 25.7 ± 16.2, and 62.8 ± 16.6 mg/L, respectively. The overall TN removal in the wetlands was 65.8%. As the wastewater from the dormitory consists mostly of water used for bathing, all NO_3-N and NO_2-N concentrations in raw water and subsequent wetland effluent were far below the USEPA maximum contaminant level standard of 10 mg/L of NO_3-N and 1 mg/L of NO_2-N and will not be further described in detail. It is clear from Fig. 6 that the three wetlands with the vegetation planted displayed better removal of TN compared with the control cell.

Figure 7 shows the variation of SRP and TP concentrations in the septic tank effluent and effluent from the four wetland cells. The mean concentration of SRP in the septic effluent was 4.0 ± 0.5 mg/L in the septic tank effluent. Mean SRP removal efficiencies of the wetland system were 99.5%, 99.0%, 99.6%, and 99.3% in wetland cells 1, 2, 3, and 4, respectively. In our case, SRP forms 71.3% of TP. The TP concentration in septic tank effluent ranged from 5.3 mg/L (September 23) to 5.7 mg/L (September 2, 2009), with a mean concentration of 5.6 ± 0.2 mg/L. The mean concentrations of TP in the effluent from four wetland cells were 0.10 ± 0.03, 0.24 ± 0.20, 0.11 ± 0.03, and 0.80 ± 0.35 mg/L, respectively. Although the vegetation uptakes a certain amount of phosphorus, known from the higher P concentration in W4 effluent, the overall TP removal in the wetlands was 99.4%, which fully demonstrated the strength of our green sorption media for phosphorus removal. The four wetland cells played a critical role in the removal of TP while having only slight fluctuations in terms of removal efficiencies.

Table 5 shows which wetland removed the most nutrient in each run and its associated removal efficiency. Wetland 1 had the highest TN and NH_3-N removal efficiency with one exception (September 9, 2009). Given the fact that NH_3-N formed about 80.4% of TN in our case, the variation of NH_3-N removal looks similar to that of TN. There was a strong

Table 4. Removal Efficiency over Four Cells of Subsurface Upflow Wetland

	Removal efficiency (%)			
Parameter	*Wetland 1 (canna)*	*Wetland 2 (blue flag)*	*Wetland 3 (bulrush)*	*Wetland 4 (control)*
FC	99.98	97.06	99.76	99.74
E. coli	100.00	99.94	99.80	100.00
BOD_5	89.4	89.6	85.2	75.2
$CBOD_5$	87.6	86.5	81.1	79.5
NH_3-N	98.6	97.9	62.0	27.6
TN	97.1	93.0	62.5	10.5
SRP	99.5	99.0	99.6	99.3
TP	98.3	95.7	98.0	85.7

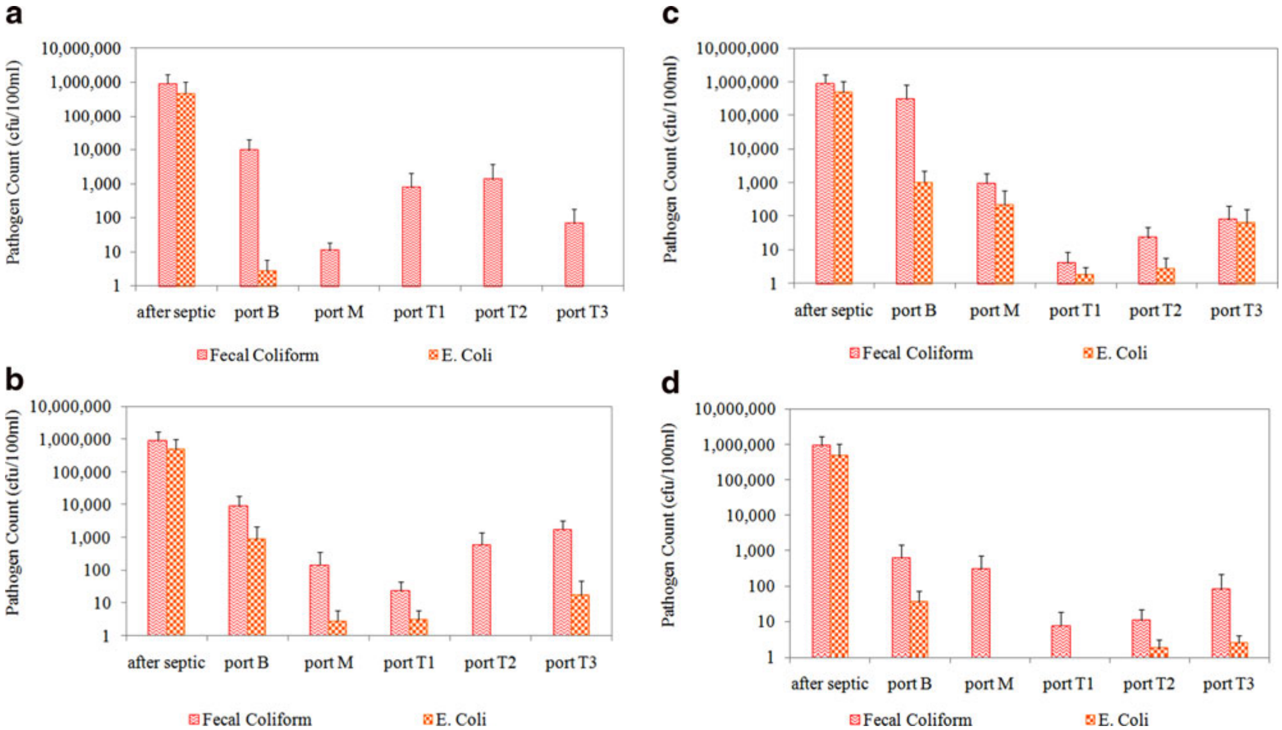

FIG. 4. Pathogen count in different cells: **(a)** wetland 1 (canna); **(b)** wetland 2 (blue flag); **(c)** wetland 3 (bulrush); **(d)** wetland 4 (control).

positive correlation between NH_3-N removal and TN removal, which verified the near complete nitrification as well as denitrification. Most NH_3-N was converted to NO_3-N, and the nitrates produced in the aerobic layer were almost completely removed within the HRT. Table 5 also shows which wetland performed best in SRP and TP removal and its removal efficiency. Occasionally, different cells displayed the best removal efficiency, as nearly 100% removal efficiency was observed in all four cells.

As mentioned earlier, the samples collected at port T3 reflected the effluent water quality and was used as a comparative basis to calculate the removal efficiencies. To gain a better understanding with respect to the flow path within the cell and the fate and transport of constituents of concern, Figs. 8 and 9 demonstrate the spatial distribution of nutrient among the four wetland cells. As expected, the concentration of all forms of N species decreased layer by layer through denitrification. But two fundamentally different trends emerged at the top layer: (1) relative stability in wetland cells 1 and 2; (2) stepwise increase from port T1 to port T3 in wetland cells 3 and 4. The N concentration patterns in cells 1 and 2 supported the design philosophy. Over 90% of N was removed along the flow pathway. On the other hand, as the result of samples from T3 was used to calculate the removal efficiency, the stepwise increase from port T1 to port T3 in cells 3 and 4 resulted in the underestimation of their performance. A short cut near the outlet or insufficient aeration could be the reason why we have such discrepancies. The removal efficiency of cells 3 and 4 can be enhanced by adding oxygenators to the outlet side or moving the outlet to the port T1. As for all forms

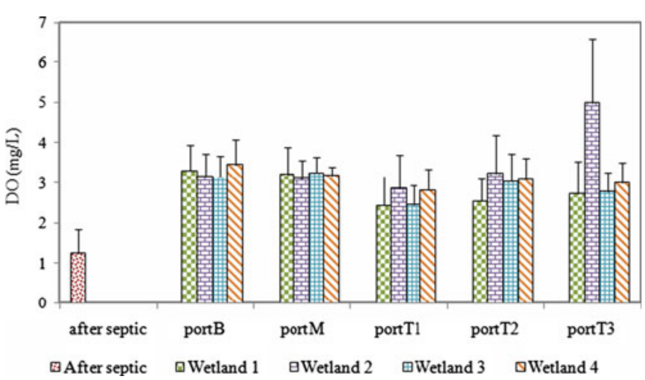

FIG. 5. Dissolved oxygen in different cells.

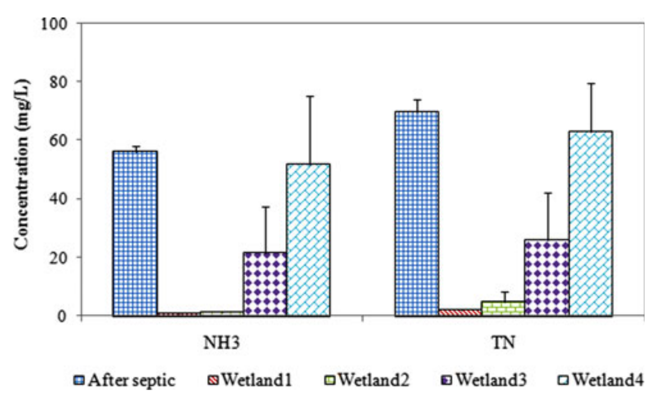

FIG. 6. Comparison of NH_3-N and TN concentrations of the septic effluent and wetland effluents. NH_3-N, ammonia-nitrogen; TN, total nitrogen.

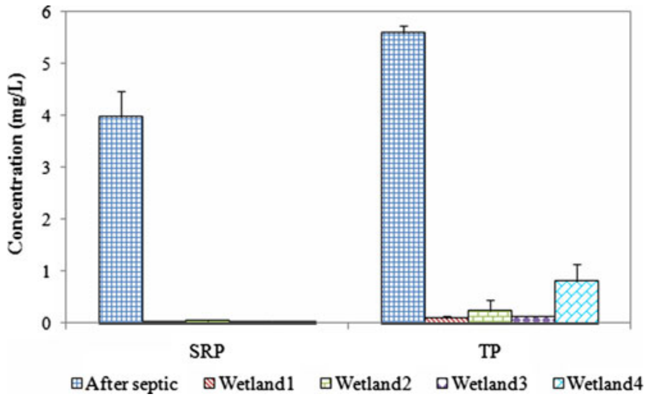

FIG. 7. Comparison of SRP and TP concentrations of the septic effluent and wetland effluents. SRP, soluble reactive phosphorus; TP, total phosphorus.

of P species, the concentrations dropped sharply except wetland cell 3, and it is evidenced that only a trace amount of P species remained after passing through the PC media.

Mass balance analyses of wetland cells

It would be worthwhile to see if the mass balance can be confirmed in each wetland cell, and if not, what is the gap? To answer such an engineering question, nutrient uptake by plants must be estimated upfront using elemental analysis equipment. Based on Table 6, the growth of all three kinds of wetland plants was obvious. Canna has a relatively broad leaf, whose fresh biomass was about 10-fold at the end of the experiment, whereas the percentage increase in fresh root biomass was almost fourfold. Bulrush with its fibrous root system received the twice increase of root biomass and an increased R:S ratio (belowground-to-aboveground biomass ratio). Blue flag had less biomass because of its tiny size. From Fig. 10, the N content in the leaves of three kinds of plants was inversely proportional to their biomass growth. Blue flag more than doubled its N content. Both blue flag and bulrush showed increased N content in leaf and decreased N content in root. P content in leaf and root of all plants decreased. Canna suffered a decrease of P content in both leaf and root. Blue flag and bulrush's P content in their roots decreased more than their leaves.

After the quantification of nutrients in plant species, the next step was to quantify the concentrations of NO_3-N and NH_4-N being absorbed on the surface of green sorption media. Figure 11 shows the NH_4-N, NO_3-N and TP concentrations in PC medium (deeper layer) and G medium from the beginning to the end of the experiment. The left part of the chart shows the initial nutrient concentration (e.g., IP1 = initial value in PC medium in wetland 1). NO_3-N remained stationary in PC medium in all four wetland cells. The NH_4-N concentration was highest in PC medium of wetlands 3 and 4, which is consistent with the water quality results. The TP concentration was highest at the end of the experiment. The stable and extremely low NH_4-N concentration in the G medium (upper layer) of wetland cells 1 and 2 reflected complete nitrification (Fig. 11b). Comparing Fig. 11a with 11b, high N and TP concentrations in wetland cells 3 and 4 were reduced by 50% in PC medium. The higher NO_3-N concentration in wetland 4 (control) implies that denitrification occurred incompletely in the PC medium without the help of wetland plants.

Figures 12 and 13 illustrate the mass balance concept of each wetland cell during the entire study period. The sum of NO_3-N and NH_4-N can be regarded as inorganic N absorbed by media. TN from the wastewater was mainly lost through denitrification (Fig. 12). In planted cells using canna and bulrush, removal of N was 4.3% and 5.3%, respectively. P uptake was <1%. For the unplanted control wetland, about 2% N was absorbed by the PC medium. After considering the pore water contribution, 80% loss of TP remains. Such observational evidence shows that the precipitation contribution is extremely large. Mineral substances in the gravel or sand layer and limestone in PC media might provide metal ions for precipitation.

Evaluation of denitrifiers' activity

To ascertain whether the denitrification was occurring in the green sorption media, this study conducted real-time PCR analysis geared toward quantifying the number of denitrifiers as an assessment index. It was shown in Fig. 14 that a certain amount of denitrifying bacteria is present in both the PC and G media. The quantity of denitrifying bacteria had a relative uniform distribution in the initial stage (blue bar). After 1 month, denitrifying bacteria in PC and G media had risen in almost all wetlands except P2. In particular, denitrifying bacteria in the PC media of wetland cell 1 increased about fivefold. Also, cell 1 had the highest amount of denitrifying bacteria in G media, which is consistent with the most satisfactory nitrogen removal efficiency of wetland cell 1. Moreover, the least amount of denitrifying bacteria exists in the G media of the control cell. It is clear that in our designed wetland, there was an anaerobic environment successfully built by the synergism of innovative upflow pattern and green sorption media. The above finding further proves that denitrification took place and was the dominant path for nitrogen removal.

Stress test

The nutrient removal ability of the SUW planted with canna has been fully confirmed in the final phase. Results of

Table 5. Comparative Performance in Each Run and Its Removal Efficiency

Sampling date	*TN*	*NH_3-N*	*TP*	*SRP*
September 2, 2009	97.4%, I	98.2%, I	98.2%, III	99.4%, III
September 9, 2009	96.6%, II	98.1%, II	97.8%, I and II	99.6%, II and III
September 16, 2009	96.6%, I	99.3%, I	98.5%, I	99.8%, IV
September 23, 2009	97.1%, I	99.5%, I	99.0%, I	99.7%, III

Roman numerals I, II, III, and IV stand for wetland cells 1, 2, 3, and 4, respectively.

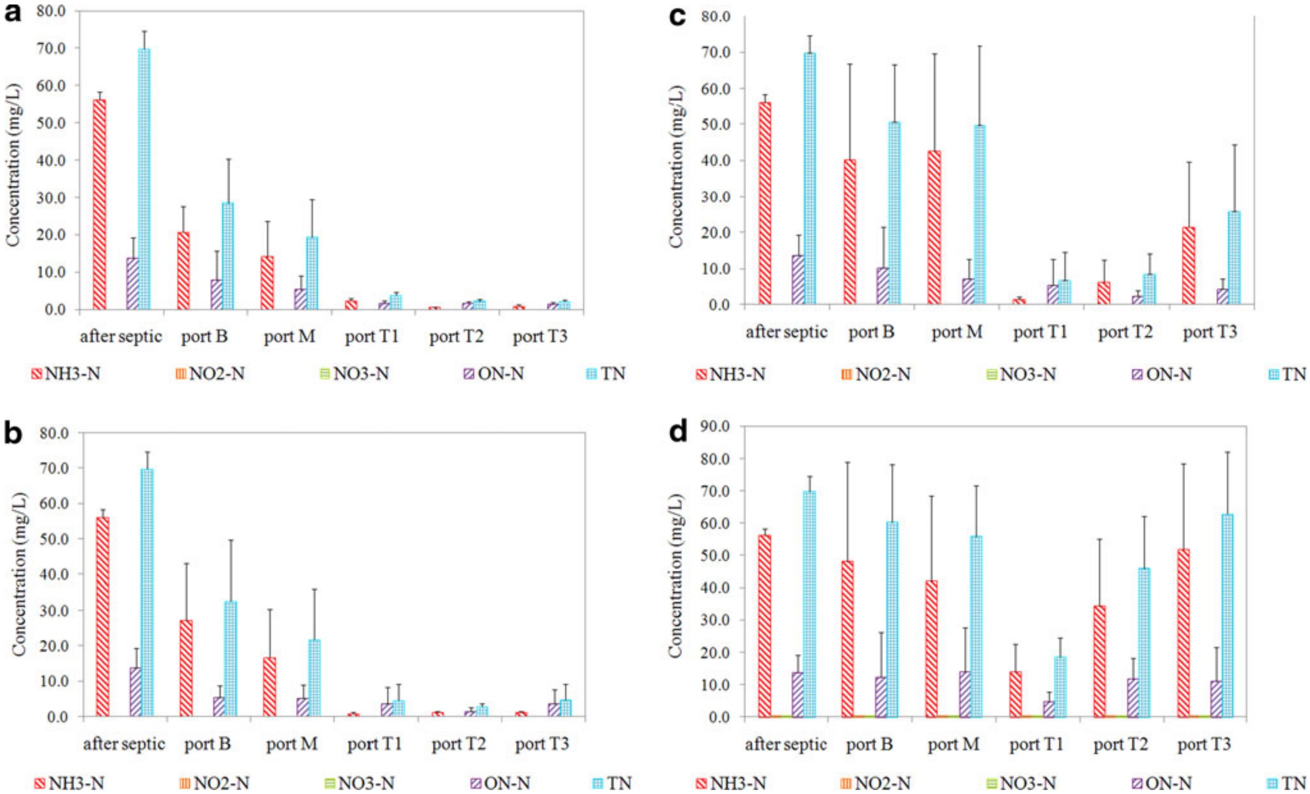

FIG. 8. Spatial distribution of nitrogen concentration: **(a)** wetland 1 (canna); **(b)** wetland 2 (blue flag); **(c)** wetland 3 (bulrush); **(d)** wetland 4 (control). NO_2-N, nitrite-nitrogen; NO_3-N, nitrate-nitrogen; ON-N, organic nitrogen-nitrogen.

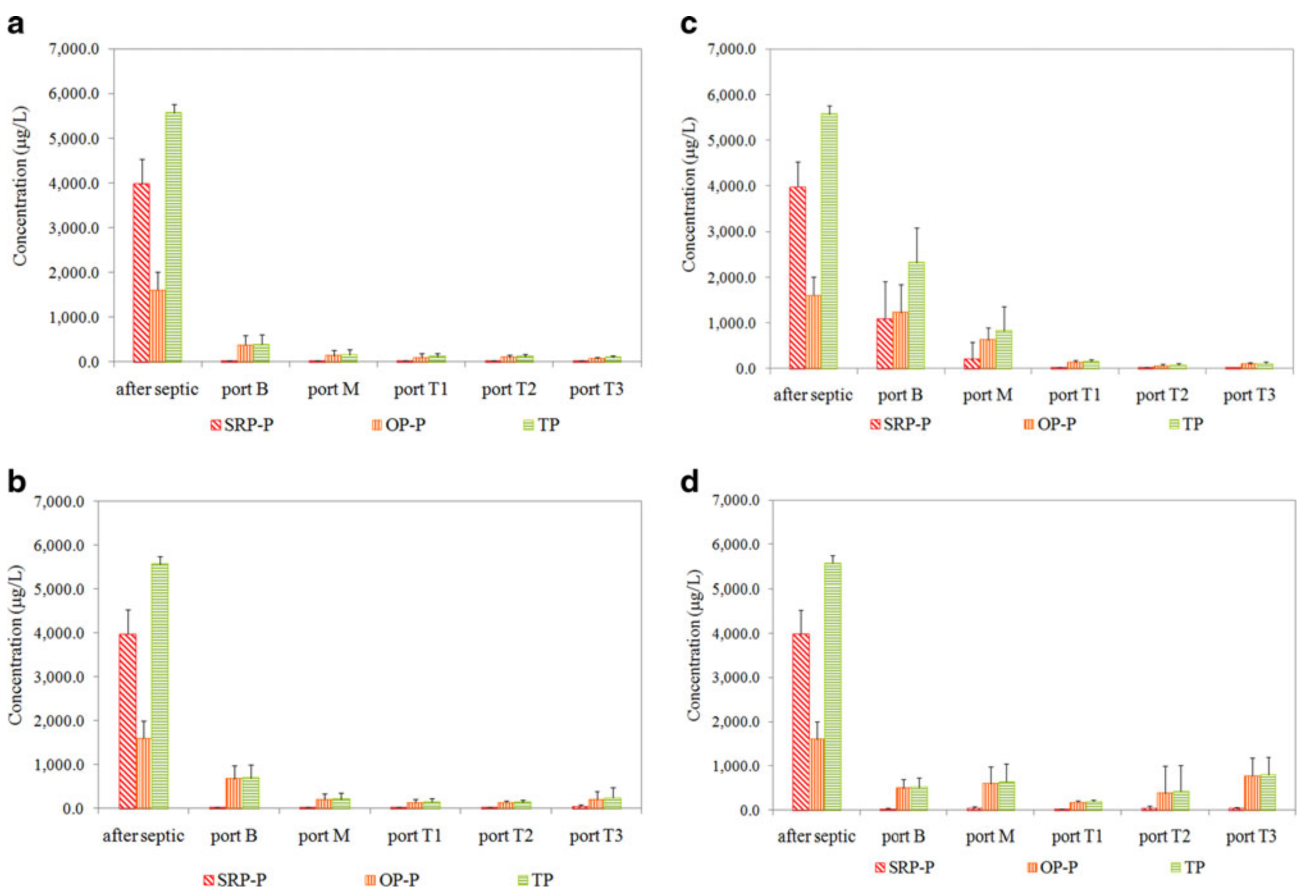

FIG. 9. Spatial distribution of phosphorus concentration: **(a)** wetland 1 (canna); **(b)** wetland 2 (blue flag); **(c)** wetland 3 (bulrush); **(d)** wetland 4 (control). OP-P, organic phosphorus-phosphorus.

Table 6. Biomass in Wetland Plant (Unit: g/Cell)

	Plant	Leaf Initial	Leaf Final	Leaf Net change	Root Initial	Root Final	Root Net change
Fresh	Canna	970	9,890	8,920	1,120	4,040	2,920
	Blue flag	295	1,185	890	699	2,015	1,316
	Bulrush	2,890	4,850	1,960	1,415	4,180	2,765
Dry	Canna	83	945	862	105	405	300
	Blue flag	41	140	99	176	275	99
	Bulrush	630	1,250	620	250	760	510

1 month of sampling indicated that it achieved a removal efficiency of 97.1% and 98.3% for TN and TP, respectively. Yet, as it is a kind of seasonal herb, canna withers in winter. Besides, the past winter (2009–2010) was reported as one of the coldest winters since records began (Fig. 15). With this the questions of "Can canna keep working without its attractive foliage?" and "What is the nutrient removal efficiency of SUW planted with canna in cold weather?" arose, and so we collected the effluent from canna SUW and compared it with control cell at the end of February, 2010.

Figure 16 demonstrates the nitrogen removal in the canna cell and control cell. About 87.4% of TN was removed in the canna cell compared with 41.0% TN removal efficiency in control cell. The higher nitrate concentration in control cell effluent showed clearly that the SUW promoted the conversion from organic nitrogen to nitrate through nitrification. In contrast, the <5 μg/L nitrate concentration in the canna cell effluent illustrated that the root system of canna still played an important role in the denitrification effect even during the severe weather condition. As denitrification is an alkalinity-producing process, the higher alkalinity in the canna cell also can be considered as a proof of successful denitrification.

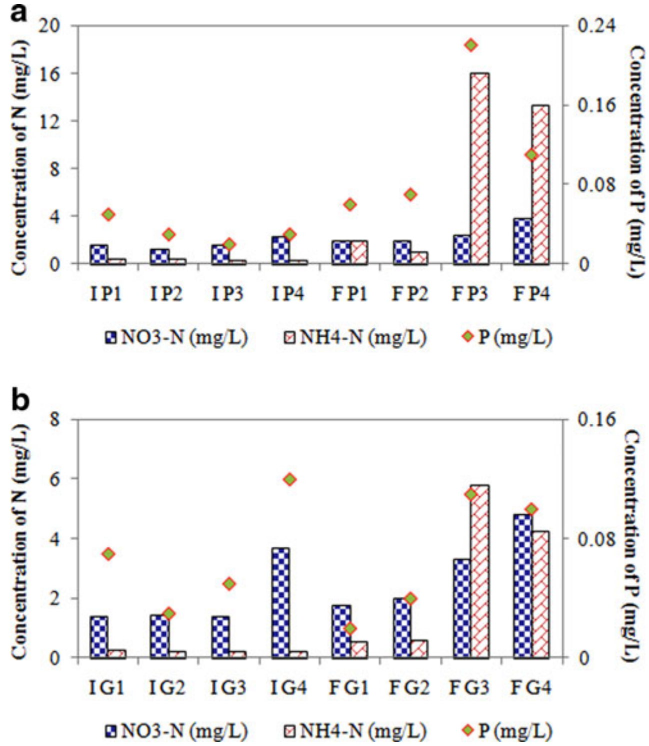

FIG. 11. NH_4-N, NO_3-N, and TP concentrations in **(a)** PC and **(b)** G medium.

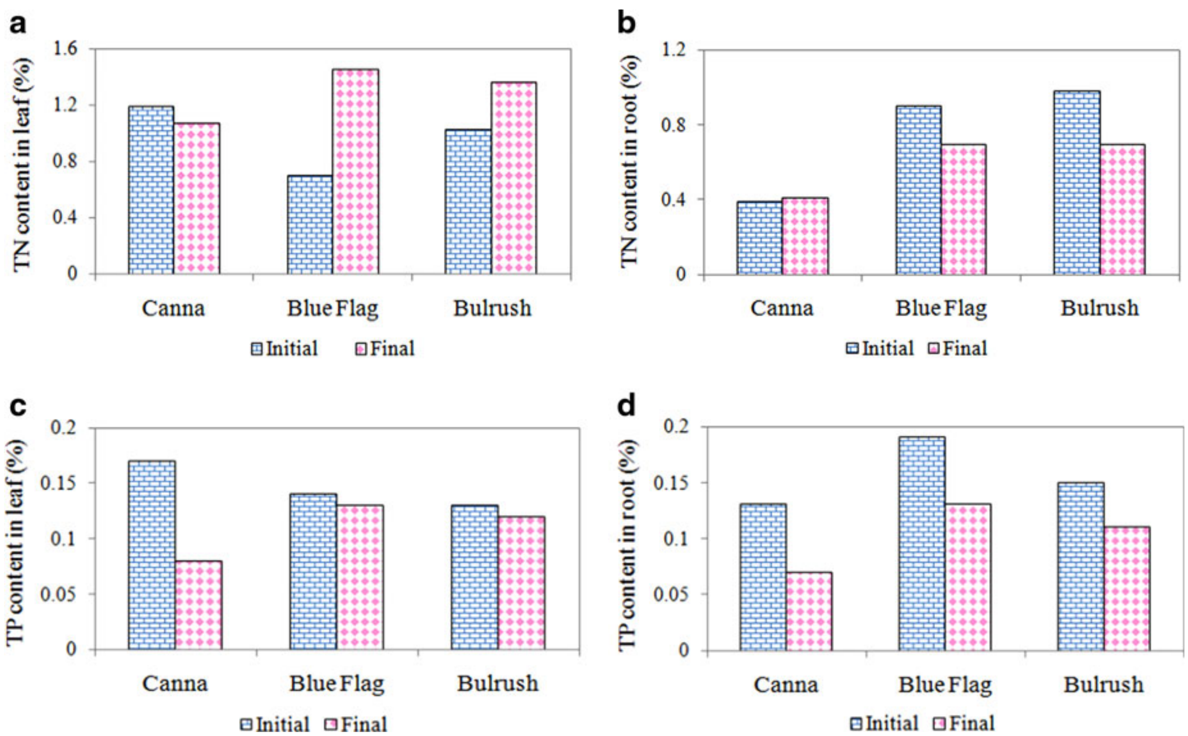

FIG. 10. Nutrient content in wetland plants: **(a)** TN in leaf; **(b)** TN in root; **(c)** TP in leaf; **(d)** TP in root.

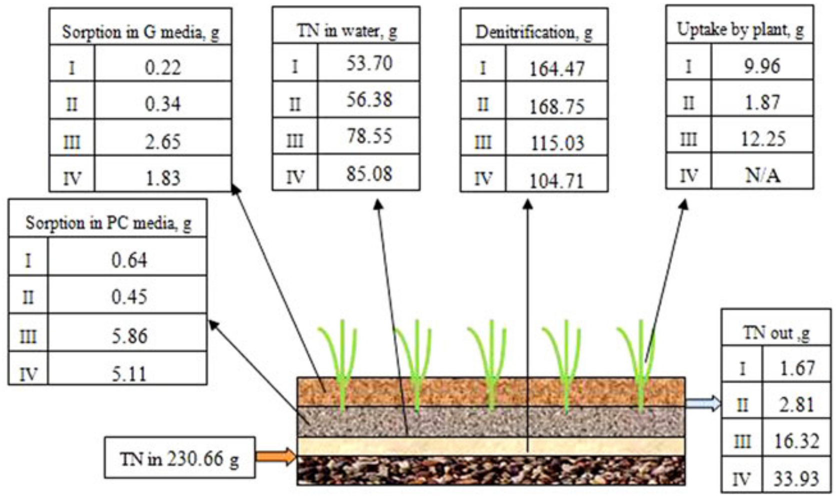

FIG. 12. Mass balance of N in four wetlands. Roman numerals I, II, III, and IV stand for wetland cells 1, 2, 3 and 4, respectively.

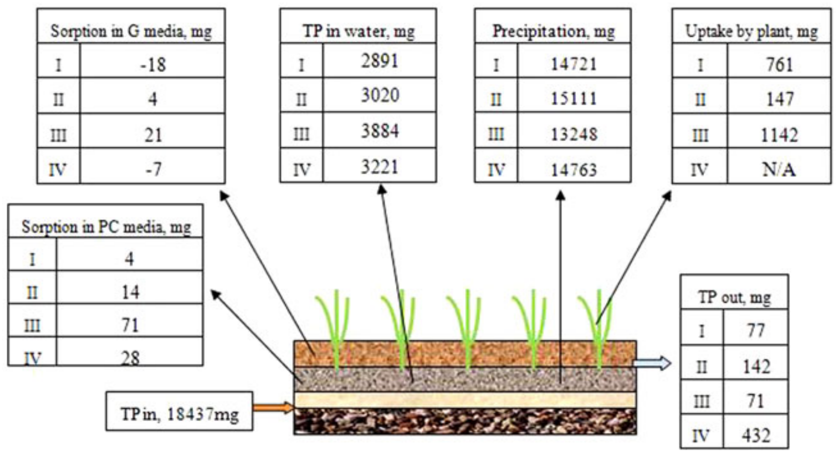

FIG. 13. Mass balance of P in four wetlands. Roman numerals I, II, III, and IV stand for wetland cells 1, 2, 3 and 4, respectively.

Figure 17 shows the difference of phosphorus removal in those two cells. Canna displayed a higher SRP removal efficiency, but a lower removal of organic P. The mean TP removal efficiency of both cells was about 98.7%. The result verified that the cold weather does not affect the TP removal in SUW. The TN removal efficiency in canna cell declined slightly during the stress test. But it still reached the level of 87.4%, which reveals that canna would be a highly competitive candidate to be planted in terms of aesthetics and nutrient control all year around.

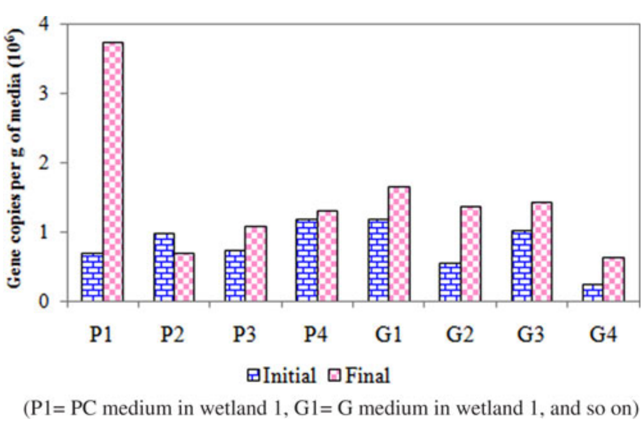

FIG. 14. Quantity of denitrifying bacteria (unit: gene copies per g of media mixture). P1, PC medium in wetland 1; G1, G medium in wetland 1; and so on.

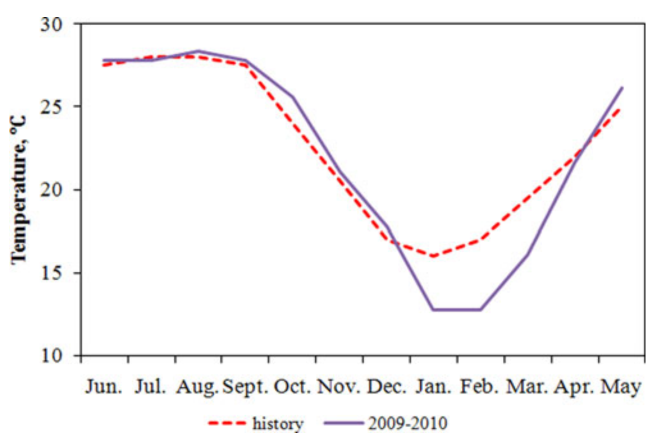

FIG. 15. Monthly temperature comparison between last year and history in Orlando.

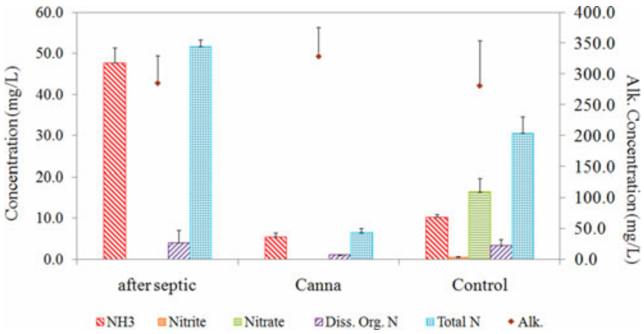

FIG. 16. Nitrogen removal in the stress test.

Conclusions

The substantial ability of the SUW in terms of removal of both nutrients and pathogens has been fully confirmed in this study. The study findings indicate that wetland 1, which was planted with canna, achieved a removal efficiency of 97.1%, 98.3%, 99.98%, and 100.0% in TN, TP, fecal coli, and *E. coli*, respectively. Canna would be a highly competitive candidate to be planted in terms of aesthetics and nutrient control all year around. Denitrification was successful as evidenced by the fact that the denitrifiers in the PC medium of wetland 1 increased even more than three times. The proposed SUW wetland technology, which is not only a good choice in terms of nutrient removal but also an outstanding device for pathogen control, is thus effective from the engineering point of view. Yet the question "what is the coupling mechanism between hydrodynamics, geochemical interactions, and microbiological activity in such upflow subsurface wetlands?" remains unanswered. A mechanistic model to address the system dynamics is expected to be the ultimate tool to answer this scientific question. Future work may focus on some advanced studies to investigate the uncertainties of ambient environment, nonideal transport of contaminants, and incomplete knowledge about sorption reactions, chemical equilibrium, and the nature of sorption or release of contaminants from media.

Acknowledgment

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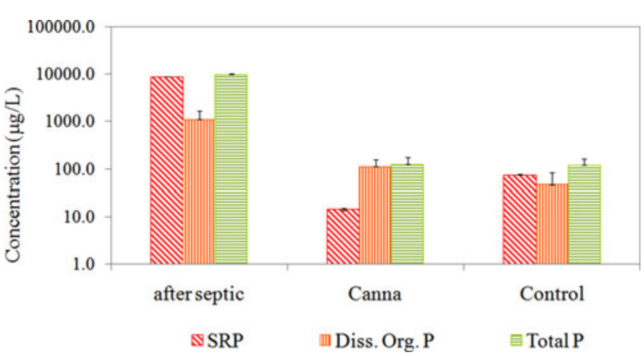

FIG. 17. Phosphorus removal in the stress test.

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