NITROGEN DYNAMICS IN A CONSTRUCTED WETLAND RECEIVING PLANT NURSERY RUNOFF IN SOUTHEASTERN UNITED STATES

By

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To every individual who nurtured my intellectual curiosity
throughout my lifetime
making this milestone possible

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Constructed wetlands are a cost effective method for on-site nutrient removal from polluted water from nonpoint sources. Nitrogen (N) concentration and denitrification potential were studied in a constructed wetland receiving plant nursery runoff over five months in southeastern US. Nursery runoff, before entering the wetland, contained an average total nitrogen concentration of 34 mg L^{-1}. Majority of nitrogen was in the form of nitrate nitrogen (NO_3^--N; 30 mg L^{-1}) followed by ammonium nitrogen (NH_4^+-N; 2 mg L^{-1}) and organic nitrogen (2 mg L^{-1}). Total phosphorus concentration was 1 mg L^{-1} and dissolved organic carbon was 11 mg L^{-1}. Mean nutrient removal efficiency of the constructed wetland was 40% for TN, 40% for NO_3^--N, 59% for NH_4^+-N, and 16% for TP. Nitrate nitrogen removal efficiency was inversely related to the loading rate of NO_3^--N; and directly related to temperature of the water column. The gradual increase in temperature over months might have enhanced NO_3^- - N removal by influencing plant growth and activity of microorganisms, which is also evidenced by the gradual decrease in the concentration of DO in the water column during the study period. The theoretical hydraulic residence time was estimated to be 3-5 days in the model. The nutrients (N and P) analyzed in this study exhibited nutrient gradient from the inflow to outflow indicating removal of these nutrients within the wetland. The denitrification potential of the water column was

relatively low (0.01 to 0.03 mg N_2O-N L^{-1} hr^{-1}), and could be due to carbon limitation in the wetland water column as evident by low DOC concentration and low DOC: NO_3^--N ratio. The presence of few attachment sites for bacteria and lack of particulate substances to support the microbial activity possibly resulted in low denitrification rates in the water column. The mean denitrification potential in the rhizosphere soil of *Typha latifolia* (3 mg N_2O-N kg^{-1} hr^{-1}) and *Canna flaccida* (4 mg N_2O-N kg^{-1} hr^{-1}) over five months were statistically similar. As the combined denitrification potential of the water column (0.72 mg N_2O-N kg^{-1} day^{-1}) and the rhizosphere soil (96 mg N_2O-N kg^{-1} day^{-1}) in this study was less than the total removal (1.75 g m^{-2} day^{-1}), it is likely that other NO_3^--N processes are contributing to the observed NO_3^--N removal in this constructed wetland. Plant uptake and microbial denitrification in the sediments and/or soil are considered as major NO_3^--N removal mechanisms in constructed wetlands. Hence, it is concluded that rhizosphere denitrification is significantly contributing to NO_3^--N removal in the constructed wetland receiving plant nursery runoff.

CHAPTER 1
INTRODUCTION

Introduction

Globally, many aquatic ecosystems have experienced an impairment of water quality due to excess nutrient loading from both point and nonpoint sources. Point source pollution refers to pollutants that come from a definite, single identifiable source. Point source pollution has been reduced effectively in recent years because point source pollutants are easy to identify, monitor, and control as compared with nonpoint source pollutants. Due to the development of new wastewater treatment technologies and implementation of stricter regulatory controls, point source pollution has effectively been reduced in the United States.

Nonpoint source pollution generally results from land runoff, precipitation, atmospheric deposition, drainage, seepage, or hydrologic modification [United States Environmental Protection Agency (USEPA), 1993a]. Nonpoint source pollutants are nutrients, metals, salts, sediments, pathogens, and toxics that come in unidentifiable runoff from agriculture, urban and suburban stormwater, mining, and oil and gas operations [National Research Council (NRC), 1992]. It is difficult to identify, control, and regulate the nonpoint source pollution, as it comes from vast and diverse landscapes, many diffuse sources, and varies by time of year. The ability of these nonpoint source pollutants to reach waterbodies is enhanced by rainfall, snowmelt, and irrigation. The nonpoint source pollutants have harmful effects on groundwater and surface water resources, drinking water supplies, recreation, fisheries, and wildlife (USEPA, 1994). The major sources of nonpoint source pollution are identified as agriculture and urban activities, which includes industry and transportation (Carpenter et al., 1998). The lack of effective control measures on polluted runoff especially from agricultural farms (including plant nurseries), urban

areas and forestry operations are the primary reason for the lack of improvement in controlling nonpoint source pollution.

Water Quality in the United States

At the beginning of the twenty-first century, nonpoint source pollution stands as the primary cause of water quality impairment within the United States. According to the USEPA (2002a), nonpoint source pollution is the main reason that approximately 39% of surveyed rivers, 45% of lakes and 51% of estuaries are not clean enough to meet basic uses such as fishing or swimming. The report from National Coastal Condition (USEPA, 2002b) projected that the 70% of the US estuary conditions would worsen by 2020 due to eutrophication caused by nonpoint source pollutants. The National Water Quality Assessment (NAWQA) program [United States Geological Survey (USGS), 1998] of the US assessed the quality of water resources, especially streams, river basins, groundwater, and aquifer systems in the Unites States from 1991-2001 and reported that the contamination of streams and groundwater is widespread in agricultural and urban areas due to nonpoint source pollutants.

States and other jurisdictions reported in the National Water Quality Inventory (NWQI, 1998) that agriculture and urban runoff are among the leading contributors to deteriorate water quality nationwide. The most common nonpoint source pollutants causing water quality impairment include nutrients [nitrogen (N) and phosphorus (P)], pesticides, chemicals, siltation (soil particles), metals, and pathogens (bacteria and viruses). According to Faeth (2000), agriculture is the largest source of pollution in the US degrading the quality of surface waters such as rivers and lakes, with croplands alone accounting for nearly 40% of the N and 30% of the P pollution. A major nonpoint source pollutant from these activities is an excess of nutrients, which can occur through applications of crop fertilizers. For example, in agricultural systems,

crops only utilize 40-60% of N fertilizer applied to fields, and the remainder is incorporated into soil organic matter, volatilized, denitrified, lost as runoff, or enters groundwater (Coffey, 1997).

Water Quality in Southeastern United States

In the Southeastern Regional Climate Assessment Report (1999), it has been reported that in many cases, water quality indices are either below recommended levels or nearly so. The major sources of nonpoint source pollution, including intense agricultural practices, urban development, coastal processes, and mining activities, impair the quality of water in the southeastern United States. In the southeastern region, especially in Georgia and Florida, the natural features such as geology, climate, hydrology, and soils, significantly influence the transport of nonpoint source pollutants from land to water. The soils of the Atlantic and Gulf coastal plains include clays, loams, and large areas of gray, sandy soils. For example, in loam/clay soils and sandy soils, the N export, as a percentage of fertilizer inputs, from agricultural systems to adjacent waterbodies ranges from 10 to 40% and 25 to 80%, respectively (Howarth et al., 1996). The transport of nutrients is also influenced by the rate, season, chemical forms and method of application, amount and timing of rainfall after application, and vegetative cover (Carpenter et al., 1998). In Florida, well drained sandy soils [underlain by gravel and carbonate (karst) rocks] are susceptible to groundwater contamination due to low water holding capacity, rapid infiltration and downward movement of water and chemicals. In contrast, the areas with poorly drained clay soils are susceptible to runoff, which leads to stream contamination, rather than groundwater contamination. Due to the poor draining capacity of the clay soils, excessive irrigation or rainfall quickly drains to the adjacent streams as runoff (USGS, 1998).

The southern coastal plain of the US holds significant agricultural and hydrological importance, with wide range of soil types and crop management systems. Land use in this region

is approximately 69% woodland, 17% cropland, 11% pastureland, and 3% urban (Berndt et al., 1996). According to the USGS (1998) report on water quality in the Georgia-Florida coastal plain during 1992-1996; "more than 20% of groundwater samples from the surficial aquifers in the agricultural areas throughout the study area had nitrate nitrogen (NO_3^- - N) concentrations greater than the USEPA drinking water standard of 10 mg L^{-1}." The concentrations of other nutrients in groundwater including ammonia, orthophosphate and total phosphorus were low. Nitrogen concentrations (as NO_3^- - N) in streams did not exceed drinking water standards or guidelines, but were higher in streams draining agricultural and mixed basins. Nearly 30% of the water samples from the streams had dissolved phosphorus concentrations greater than the aquatic criteria (0.1 mg L^{-1}; USEPA, 1986) for total phosphorus. Nearly 80% of the streams in agricultural areas had phosphorus concentrations greater than the USEPA standard to prevent algal growth (USGS 1998). The study area, situated in the Georgia-Florida coastal plain, overlies the Upper Floridan aquifer, which is the major source of drinking water for that area. The unconfined Upper Floridan aquifer has karst features, which is vulnerable to groundwater contamination, similar to that of sandy soils (USGS, 1998).

Water Quality and Ornamental Plant Industry in Georgia and Florida

Florida is one of America's leading agricultural states which produces a wide range of commodities. The state has a humid subtropical climate with mean rainfall of up to 140 cm per year, mean monthly temperature ranging from 4 to 33˚C during a calendar year, and is prone to hurricanes (Black, 1993). Florida has the largest total acreage of 'Aquods', (wet sandy soils with an organic stained subsoil layer; www.nrcs.gov, November 2007), are susceptible to groundwater contamination due to poor water holding capacity. Hence, pollution of surface and groundwater resources resulting from agricultural runoff, urban stormwater runoff, and erosion

sedimentation is a significant problem in Florida, where approximately one-third of landforms are wetlands, and drinking water is mostly drawn from underground aquifers which are close to the land surface (Marella and Fanning, 1996).

The generally temperate climate in Georgia is influenced by the proximity to the Atlantic Ocean and to the Gulf of Mexico. The coastal plains, underlain by sand and limestone, occupy over 60% of the state. Because of the sandy soil and flat topography in the coastal areas, infiltration is a larger concern rather than runoff. Nearly one third of the state is underlain by sandy/clay loam soils, which support variety of agricultural crops. The state typically receives 100-125 cm of rainfall every year (www.georgia.org; www.coastgis.marsci.uga.edu, October 2007).

Florida is the second leading producer of ornamental plants in the US. The greenhouse and nursery industry comprises 50,992 hectares of production area in Florida (Hodges and Haydu, 2002). According to the United States Department of Agriculture (USDA) estimates, the value of nursery and greenhouse crops in Florida was approximately $1.63 billion in 2004 (Jerardo, 2005). Georgia is among the top ten producers of floriculture crops, ranking tenth in the US. The value of nursery and greenhouse crops in Georgia was approximately $400 million in 2006 [National Agricultural Statistics Service (NASS), http://www.nass.usda.gov/, accessed on October, 2007].

While greenhouse crop production generates significant runoff, this problem is most noticeable in the container nursery industry where large numbers of containerized plants are grown outdoors. Generally, for production of containerized plants outdoors, plants are grown in containers placed on gravel beds which are lined with plastic to prevent weed growth, and irrigation is achieved through overhead sprinklers (USDA, 1998a; Lea-cox and Ross, 2001). The

ornamental nursery production requires intensive irrigation and fertilization for production of marketable plants. While many growers use controlled release fertilizers (CRFs) incorporated into the medium, liquid fertilizers are also applied through overhead irrigation systems at several operations. The method of fertilizer application has been shown to influence the concentration of nutrients in the runoff. The CRFs are observed to be suitable to minimize the concentration of nutrients in the runoff. For example, NO_3^- - N concentration was observed to be low (0.5 to 33 mg L^{-1}, averaging 8 mg L^{-1}) when CRFs were used, as compared to 0.1 to 135 mg L^{-1} (averaging 20 mg L^{-1}) when a combination of CRFs and liquid fertilizers was used (Yeager et al., 1993). The liquid fertilizers are typically used because CRFs are often more expensive and do not provide the nutrients as readily as the liquid formulations. Nitrate fertilizers dissolve readily, they are highly mobile in the water, hence leach readily, and are often found in the runoff water. Compared to NO_3^- - N, phosphorus is rapidly retained as insoluble inorganic compounds and sorbed to soil surfaces. Overhead irrigation is the primary method of applying irrigation to ornamental plants in small containers. The irrigation application efficiency is reported to be low, ranging from 15% to 30% (Lu and Sibley, 2006). The overhead sprinkler irrigation of 1.8×10^5 L ha^{-1} yr^{-1} (Aldrich and Bartok, 1994) is estimated to produce $1.8 - 9 \times 10^4$ L ha^{-1} yr^{-1} of wastewater as runoff (Berghage et al., 1999) accounting for 10-50% loss as runoff of the irrigation water. The irrigation practices lead to runoff of excessive fertilizers which find their way into nearby water resources either through percolation in case of bare ground or through runoff in case of plastic covered nursery beds. Due to high amount of rainfall and soil conditions in Florida and Georgia, there is significant potential for discharge of large volume of runoff with high nutrient concentration to nearby surface and groundwater resources. Hence runoff from plant nursery poses a threat to water quality in Florida and Georgia. The restrictions/legislations

such as Watershed Restoration Act and Total Maximum Daily Load (TMDLs), imposed by the environmental protection agencies against contamination of water resources necessitate recycling/treatment of nursery runoff before discharging into nearby waterbodies (Headley et al., 2001; Lea-cox et al., 2002; Huett et al., 2005).

Management Strategies to Control Nonpoint Source Pollution

The control of nonpoint source pollution centers on adoption of Best Management Practices (BMPs) to manage land and to control the release of pollutants into the atmosphere; further, establishment of threshold levels of contaminants (e.g., TMDLs) is also a strategy for limiting nonpoint source pollution (Carpenter et al., 1998).

Best Management Practices can be grouped into two categories; structural and nonstructural (Horner et al., 1994). Non structural BMPs include practices such as preservation of natural areas and drainage systems, land use methods, and efficient use of fertilizers and irrigation water. Structural BMPs include establishment of detention/retention and recycling ponds, grass swales, filter strips, riparian buffer strips, floodplains, and wetlands. The commonly used structural BMPs are retention and recycling ponds, riparian buffers strips, and grass swales to control nonpoint source pollution from agricultural operations, including plant nurseries. In most of these methods, the primary mechanisms to remove pollutants are to enhance settling of the particulates and to enhance infiltration into the subsurface zones. Many container plant nurseries, where plants are grown outdoors on gravel beds lined with plastic, construct recycling ponds to recycle the runoff from nursery beds (Lu and Sibley, 2006). The other commonly followed BMPs to control the runoff water from container plant nurseries are adjusting the time and rate of fertilizer application to coincide with the crop needs, using micro-irrigation practices to minimize runoff volume, and use of controlled release fertilizers to reduce NO_3^- - N leaching from containers.

A TMDL specifies the maximum amount of a pollutant that a water body can receive and still meet water quality standards, and establishes pollutant loadings among point and nonpoint pollutant sources (www.epa.gov, October 2007). The 1999 Florida Legislature adopted comprehensive TMDL legislation, and concluded that the development of a TMDL program will promote improvements in water quality throughout the state through the coordinated control of point and nonpoint sources of pollution. "The scientifically based TMDL program was found to be necessary to fairly and equitably allocate pollution loads to both nonpoint and point sources", (Thomas and Joyner, 2002). In Georgia, over 850 TMDLs had been approved by the end of 2002 by Georgia Environmental Protection Division and USEPA. Most of the TMDLs in Georgia, however, were established for fecal coliform bacteria (pathogens), metals, sediments, and dissolved oxygen (http://pubs.caes.uga.edu/caespubs/pubs/PDF/B1242-2.pdf, October 2007).

Importance of Natural and Constructed Wetlands for Improving Water Quality

Wetlands and forested riparian buffers are most commonly utilized management strategies for on-site nutrient removal from polluted waters generated from agricultural practices, including plant nurseries (Franklin et al., 2000). Among these, wetlands can play an important role in ecosystem management and can act as a sink for nutrients. Wetlands, however, often have been destroyed for agricultural use of land and for urban development. In the United States alone, approximately 54% of the original wetlands have been destroyed (Patrick, 1994). By the mid-1980's, Florida had lost approximately 46% of its estimated original wetlands (Dahl, 1990). Georgia had lost approximately 23% of its estimated original wetlands, by the mid 1980's (Dahl, 1990). However, in the past few decades, planned use of natural wetlands for treating wastewater treatment has been seriously studied and implemented in a controlled manner (USEPA, 1993b). Gren (1995) reported that wetland restoration is the most cost effective method of decreasing nonpoint source pollution.

Over the recent years, it has been realized that in addition to restoring the natural wetlands, it is useful to construct artificial wetlands for cost effective, on-site remediation of polluted water before discharging it into the natural aquatic systems. A number of studies have provided evidence that constructed wetland systems provide an effective means to control the nonpoint source pollution (including nutrient contaminants), thereby improving water quality (Mitsch, 1992; Hammer, 1992; Kadlec and Knight, 1996; Hoag, 1997; Headley et al., 2001; Poe et al., 2003; Huett et al., 2005; Yang et al., 2007). Constructed wetlands are engineered systems that differ from natural systems but are used to treat water by mimicking the processes occurring in natural wetlands. These are designed to provide an ideal habitat for microbial communities for achieving nutrient transformations in the contaminated water (Kadlec and Knight, 1996). Wetland soil is highly saturated and exists in a chemically reduced state. The oxidized forms of nutrients undergo transformations when it come in contact with saturated and reduced soil conditions. Biological activity in the biofilms that attach to wetland soil and plants accounts for much of the transformations of pollutants. Nitrogen transformations such as nitrification and denitrification in the sediments were observed to occur rapidly in the aerobic/anaerobic microsites in the wetlands (Kadlec and Knight, 1996).

Constructed wetlands are increasingly being used for treating N rich wastewater (Gersberg et al., 1983; Reddy and D'Angelo, 1994; Bachand and Horne, 2000; Headley et al., 2001; Poe et al., 2003; Huett et al., 2005; Reinhardt et al., 2006; Yang et al., 2007). Such systems have been subjected to wastewater discharges from municipal, industrial, agricultural and surface runoff, irrigation return flows, urban stormwater discharges, and other sources of polluted water. The constructed wetland systems can be broadly classified into two main categories; surface flow and subsurface flow systems. The surface flow wetlands are designed to have a shallow (typically

less than 0.4m) layer of surface water over mineral or organic soils. The persistent emergent vegetation such as *Typha* spp.L. (Cattail), *Phragmites* spp. Adans. (Reed), and *Scirpus* spp. L. (Bulrush) are commonly used, along with floating and submerged aquatic vegetation, and shrubs and trees. In subsurface wetlands, the basin (less than 0.6m) is filled with coarse substrate such as gravel, and the water level is maintained below ground with water flowing through the gravel and the roots of vegetation (DeBusk, 1999). Both surface flow and subsurface flow constructed wetlands have been reported to remove N and P from plant nursery runoff (Taylor et al., 2006). A surface flow wetland was reported to remove 79-99% of nitrogen from the plant nursery runoff (Taylor et al., 2006).

Nitrogen Cycling in Wetlands

Constructed wetlands are recognized as a means to improve water quality through N removal. The major contributors of organic and inorganic N to the constructed wetlands include biological N fixation and point and nonpoint source runoff, respectively. Organic N undergoes mineralization/decomposition and results in the release of inorganic N forms. The processes such as biological nitrification, denitrification, and volatilization result in the production of inorganic N forms (ammonia, nitrate, nitrite and N_2 gas).

Most of the N transformations in the constructed wetlands are either plant or microbe mediated. Nitrification is an aerobic process, in which oxidation of ammonium nitrogen (NH_4^+-N) to nitrate nitrogen (NO_3^- - N) is mediated by nitrifying bacteria. In contrast, denitrification occurs in anaerobic conditions, whereby NO_3^- - N is reduced to N_2 gas. There is growing evidence that other microbe mediated processes such as Dissimilatory Nitrate Reduction to Ammonium (DNRA) and anaerobic ammonium oxidation (anammox) facilitate N transformations in wetlands (Stottmeister et al., 2003; Shipin et al., 2005; Reddy and Delaune, 2007; Burgin and Hamilton, 2007; Paredes et al., 2007).

The temporary removal of N in wetlands occurs through plant uptake and microbial immobilization, whereas, permanent removal of N occurs through denitrification (Fig. 1-1). In wetlands, biological denitrification is the major pathway of N removal (Reddy et al., 1989; Tanner and Kadlec, 2003; Poe et al., 2003; Toet et al., 2003; Smialek et al., 2006). At high pH (typically higher than 8.0) volatilization of ammonia also contributes to N loss to the atmosphere (Reddy and Patrick, 1984). Other processes such as sedimentation, adsorption, uptake by plants and microbes lead to N storage in the wetlands (Fig. 1-1).

Denitrification in Wetlands

Denitrification is the stepwise reduction of nitrogenous oxides (NO_3^- and NO_2^-) to produce gaseous N products (N_2O and N_2). These gases are released into the atmosphere, thus resulting in a removal of N from the wetland. It is a microbe mediated process in which facultative anaerobic bacteria (*Pseudomonas* spp., *Alcaligenes* spp., *Flavobacterium* spp., *Paracoccus* spp., and *Bacillus* spp.) use nitrate (NO_3^-) as the terminal electron acceptor during the oxidation of carbon (C) in the absence of oxygen (O_2) (Tiedje, 1988). The low redox potential and high carbon content in the wetlands favors the process of denitrification. Globally, N loss due to denitrification process is estimated as 19 Tg yr^{-1} (Armentano and Verhoeven, 1990). The equation and the chemical transformations of denitrification process are as follows

$$2NO_3^- + 10e^- + 12H^+ \longrightarrow N_2 + 6H_2O$$

Nitrate (NO_3^-) ⟶ Nitrite (NO_2^-) ⟶ Nitrous oxide (N_2O) ⟶ Nitrogen (N_2)

The microbe mediated denitrification process was observed to be the dominant mechanism for N removal in the constructed wetlands (Xue et al., 1999; Bachand and Horne 2000). Hence the quantification of denitrification potential is critical. The commonly used methods are mass balance, Acetylene Inhibition Technique (AIT), ^{15}N tracers, and Membrane Inlet Mass Spectrometry (MIMS). Due to the differences in the methodology, the comparison of

denitrification rates between the studies is often challenging (Seitzinger, 1993). The AIT method is one of the most commonly used techniques for measuring denitrification rates. This technique measures the denitrification rates under ambient anaerobic conditions, in which acetylene (C_2H_2) inhibits the reduction of N_2O to N. The extent of denitrification is quantified by the progressive accumulation of N_2O. This is also known as Denitrification enzyme Assay (DEA), which is an indicator of denitrifier biomass present in the samples and serves as an integrated measure of denitrification potential.

Factors Influencing Denitrification in Wetlands

Denitrification in wetland systems is primarily controlled by organic carbon availability (Reddy et al., 1982; Gale et al., 1993; D'Angelo and Reddy, 1999), aeration status (dissolved oxygen levels; Tanner and Kadlec, 2003), and NO_3^- - N concentration (Cooper and Findlater, 1990; Gale et al., 1993; Martin and Reddy, 1997). These factors are highly influenced by temporal (diurnal and seasonal) and spatial (horizontal and vertical) variation (White and Reddy, 1999; Kadlec and Reddy, 2001). The other factors that influence denitrification rates in wetlands are the make-up of the vegetative and microorganismal communities (Gersberg et al., 1986; Sirivedhin and Gray, 2006; Smialek et al., 2006), depth of the water column (Sirivedhin and Gray, 2006), hydraulic residence time, and pH of the water and soil (Reddy and Patrick, 1984).

The major regulator of denitrification is the oxygen status of the wetland, as this process is facilitated by facultative anaerobic bacteria. Denitrification is observed to take place only in low oxygen zones of the wetlands (Knowles, 1982; Knowles, 1990; Tiedje, 1988). In anoxic conditions, denitrification is controlled by NO_3^- - N and carbon availability.

The importance of organic carbon as an electron donor in the denitrification process is well documented (Eriksson and Weisner, 1997; Lin et al., 2002; Bastviken et al., 2003). While studying the relationship between denitrification rates and dissolved organic carbon (DOC),

significant positive correlation was observed by Reddy et al. (1982) and Gale et al. (1993) for a wide range of wetlands. The positive linear relationship indicates there is direct relationship between denitrification and DOC, i.e. denitrification increases with increasing DOC concentrations. Hence the denitrification rate is related to the rate of mineralization of C and level of bio-available C to the denitrifying microbes (Reddy et al., 1982; Gale et al., 1993).

Martin and Reddy (1997) suggested that denitrification rates are limited by NO_3^- - N concentrations rather than C availability and by diffusion rates of NO_3^- - N from aerobic to anaerobic phase in wetlands. Many researchers observed positive correlation between NO_3^- - N concentrations and denitrification rates (White and Reddy 1999; Sartoris et al., 2000; Gale et al., 1993; Poe et al., 2003). Responses of denitrification rates to changes in NO_3^- - N concentrations between 0.7 – 10.5 mg L^{-1} were assessed (by NO_3^- - N addition experiments) and observed significant correlation between nitrate concentration and denitrification rates (Poe et al., 2003).

The nutrient gradient from the inlet to outlet points and along the soil depth in a constructed wetland is reported to influence the denitrification rates (White and Reddy, 1999; White and Reddy, 2003; Sirivedhin and Gray, 2006). Increased denitrification rates were observed at the inflow of the wetlands where there was high NO_3^- - N concentration as compared to the outflow, where there was low NO_3^- - N concentration. The denitrification potential decreased exponentially with increasing distance, due to decreasing NO_3^- - N concentration, from inflow point to the outflow point (White and Reddy, 1999). Similar results of increased denitrification potential near the inlets and decreased denitrification potential in the middle and outlet of the constructed wetlands were observed by Sirivedhin and Gray (2006). The higher denitrification potential was observed in the surface soil, between 0-15 cm, (averaging 14.1 mg

N_2O-N kg^{-1} h^{-1}) as compared to 0.51 mg N_2O-N kg^{-1} h^{-1} in the underlying soil, between 10-30 cm, of the wetlands.

The residence time of water in a wetland is critical for removal of N because it affects the duration of the contact between polluted water and the biotic and abiotic components such as plant roots, soil, sediments, and microbes. The longer residence time may enhance removal of N by promoting retention and biochemical processes such as denitrification, sedimentation, and plant uptake (Kadlec and Knight, 1996; Mitsch and Gosselink, 2000). Kadlec and Knight (1996) also showed that the N removal efficiency is logarithmically related to the residence time.

Temperature is a significant abiotic factor that influences microbial activities. Wood et al. (1999) reported that the temperature influences the biological and physical activities in the wetlands, and denitrification is a temperature dependent process. The rates of denitrification have been shown to increase by 1.5-2 times with each increase in 10oC (Reddy and Patrick, 1984). The denitrification rates were higher (9.2 mg N m-2 h-1) in summer and lower (0.7 mg N m-2 h-1) in fall in the constructed wetlands (Poe et al., 2003); and similarly Xue et al.(1999) reported higher denitrification rates in summer (11.8 mg N m-2 h-1) and lower (2.0 mg N m-2 h-1) in winter. White and Reddy (1999) reported highest (2.69 mg N2O-N kg-1 h-1) activity of denitrifying enzymes during the summer when the temperature, hydraulic loading, and nutrient loading were highest. Spieles and Mitsch (2000) reported the optimum range of temperature for denitrification in wetland sediments as 20 to 25 ^{0}C. Water temperatures less than 15°C or greater than 30°C have been shown to limit the rate of denitrification (Reddy and Patrick, 1984). The most effective pH for denitrification ranges from 7.0 to 8.5, with the optimum at 7.0 (Reddy et al., 2000).

Denitrification occurs in the anoxic water column of aquatic ecosystems. Many researchers observed lower significant denitrification potential activity in the water column as compared to the sediments in different aquatic environments. The denitrification rates of the water samples in the wetland receiving sewage treatment plant effluent were observed to be 0.06 – 0.96 μg N L^{-1} hr^{-1} (Toet et al., 2003). Bastviken et al. (2003) obtained higher denitrification rates (6.5-7.5 kg N ha^{-1} day^{-1}) in the sediment than on surface of the water column.

Aquatic plants serve as a major source of organic carbon to the microorganisms and provide large surface area (commonly referred as "biofilms") for nitrifying and denitrifying bacteria. Plants also provide oxygen, which vary from species to species, to their root zones (Stoottmeister et al., 2003). Diffusion of oxygen to root zones is reported to influence biogeochemical cycles within the rhizosphere. But it was shown that the amount of oxygen being released by the plants around the roots is limited (Brix, 1994; Wood, 1995). The limited release of oxygen around roots ensures that anaerobic conditions will predominate unless the organic load to the wetland is low and wetland is shallow, as the amount of oxygen decreases with increasing depth (Ayaz and Acka, 2000).The limited release of oxygen through roots, however, also provides the required oxygen for aerobic nitrifying microbes for the oxidation of NH_4^+-N to NO_3^--N/nitrite nitrogen (NO_2^--N), which in turn provides nitrate/nitrite for the anaerobic denitrifying microbes for denitrification (Risgaard-Petersen and Jenson, 1997). The oxygen release rates of floating and emergent aquatic plants such as *Pistia stratiotes* L. (water lettuce), *Eichhornia crassipes* (Mart.) Solms (water hyacinth), *Hydrocotyle umbellata* L.(pennywort), *Pontederia* spp.L. (pickerel weed), *Phragmites* spp. (Reed) and *Typha latifolia* L. (cattail) were studied by Moorhead and Reddy (1988); the oxygen release rate in *Phragmites* spp. was estimated to be from 0.02 g m^{-2} day^{-1} to 12 g m^{-2} day^{-1}. Reddy et al. (1989) studied the

effectiveness of three floating and six emergent aquatic plants in improving domestic wastewater quality based on their oxygen release capacities. Plants such as *H. umbellata* transported oxygen 2.5 times more rapidly than *E. crassipes*, which transports oxygen four times more rapidly than *P. stratiotes*. Radial oxygen loss (ROL), which is influenced by the external oxygen demand, was higher in *Juncus effusus* L. (common bulrush) ($9.5 \pm 1 \times 10^{-7}$ mol O_2 h^{-1} $root^{-1}$) than in *J. inflexus* L. (inland bulrush) ($4.5 \pm 0.5 \times 10^{-7}$ mol O_2 h^{-1} $root^{-1}$) (Sorrell, 1999). Higher rates of (6.8 mg N m^{-2} d^{-1}) denitrification were obtained in the wetland sediments planted with *Juncus* spp as compared to the unplanted plots (Smialek et al., 2006). This indicates the importance of vegetation in the constructed wetlands to facilitate denitrification.

Besides oxygen, plant roots also exude organic compounds, which serve as a carbon source for denitrifying microorganisms. The influence of temperature on plant metabolism in turn affects the concentration of root exudates. The magnitude of organic compound release, which enhances the NO_3^- - N removal in constructed wetlands, ranged from 5-25% of the photosynthetically fixed carbon (Platzer, 1996). Bachand and Horne (2000) observed that the different vegetation types resulted in significantly different denitrification rates (*T.latifolia* 565 mg N m^{-2} day^{-1}, *J. effusus* 261 mg N m^{-2} day^{-1}, and mixed vegetation 835 mg N m^{-2} day^{-1}). Productivity, physical structure, $C:N_{litter}$ ratio, and plant fiber content have been observed to differ between plants. Hence they concluded that these factors affected the organic carbon availability of plants, thereby resulting in different denitrification rates. In organic carbon limited, free surface wetlands, it was recommended that a mixture of labile (submergent and floating) and more recalcitrant (emergent and grasses) vegetation be planted to enhance denitrification rates (Bachand and Horne, 2000).

The Problem Statement

Among the various management strategies developed to mitigate nonpoint source pollution, constructed wetlands are considered as a cost effective method for on-site removal of nutrients from nonpoint source pollution. The performance of the constructed wetlands varies with site, characteristics of the wastewater, type and design of the wetland. Hence, a "systems approach" which recognizes site specific conditions, is essential for the successful management of the plant nursery runoff (Mitsch and Jorgensen 1989; NRCS, 2002; Dunne et al., 2005). Most research has focused on use of constructed wetlands to treat wastewater from municipal water source, animal waste, urban runoff, stormwater runoff, and agricultural runoff. Very few studies have focused on the constructed wetlands treating nursery runoff (Headley et al., 2001; Lea-cox et al., 2002; Huett et al., 2005). Constructed wetlands that receive nursery runoff show considerable difference in water quality as compared with other wastewater sources. One of the main differences is the low concentration of dissolved organic carbon (DOC) in the nursery runoff (Huett et al., 2005), which is due to type of medium used in the containerized plants. Nursery runoff can have seasonal and hydraulic variability based on the timing of fertilizer application and rainfall. Due to the characteristic features of the plant nursery runoff, it is important to understand the nutrient removal mechanisms in a constructed wetland treating nursery runoff.

One of the methods to increase the efficiency of nutrient removal is to implement management and design practices to increase the denitrification process in constructed wetlands. Although denitrification is considered a major removal mechanism for N in constructed wetlands, the importance of this process in wetlands receiving plant nursery runoff has not been studied. Earlier studies (Bachand and Horne, 2000) indicated that different vegetation types resulted in different denitrification rates in the wetlands which could be attributed to the

differences in the availability of organic carbon in the respective macrocosms. The influence of different macrophyte rhizosphere on denitrification rates within the constructed wetlands receiving plant nursery runoff is not known. The goal of this study was to assess the N dynamics in a constructed wetland receiving plant nursery runoff for on-site N removal. To achieve this goal, following specific objectives and hypotheses were addressed.

Objectives and Hypotheses

- **Objective 1:** To determine the N and P removal efficiency of a constructed wetland receiving plant nursery runoff.

- **Hypothesis 1:** The concentration of N and P will vary over months based on the timing of fertilizer application at the nursery, irrigation, and rainfall. The concentration of N and P will be higher at inflow as compared to the outflow water, due to the nutrient removal within the constructed wetlands.

- **Objective 2:** To theoretically model the effect of hydraulic loading rate of the runoff and of estimated plant density on the residence time, which influences N removal in a constructed wetland.

- **Hypothesis 2:** The theoretical residence time will increase with increasing plant density and decreasing loading rate.

- **Objective 3:** To assess the concentration of nutrients (N and P) and denitrification potential within the water column at varying depths (surface and bottom) and variation associated with the locations across the wetland (inflow and outflow) over five months in a constructed wetland receiving plant nursery runoff.

- **Hypothesis 3:** The concentration of nutrients (N and P) will be higher at inflow as compared to the outflow. The denitrification potential will be higher in the water samples collected from the lower depths as compared to the surface of the water column due to the anoxic conditions in the deeper parts of the water column.

- **Objective 4:** To determine the denitrification potential in the rhizosphere soil (soil closely adhering to the roots) of *Canna flaccida* and *Typha latifolia* in a constructed wetland receiving plant nursery runoff.

- **Hypothesis 4:** The denitrification potential of the rhizosphere soil (soil closely adhering to the roots) will vary from species to species because macrophytes can vary in their capacity to diffuse oxygen and different carbon compounds in their root zones.

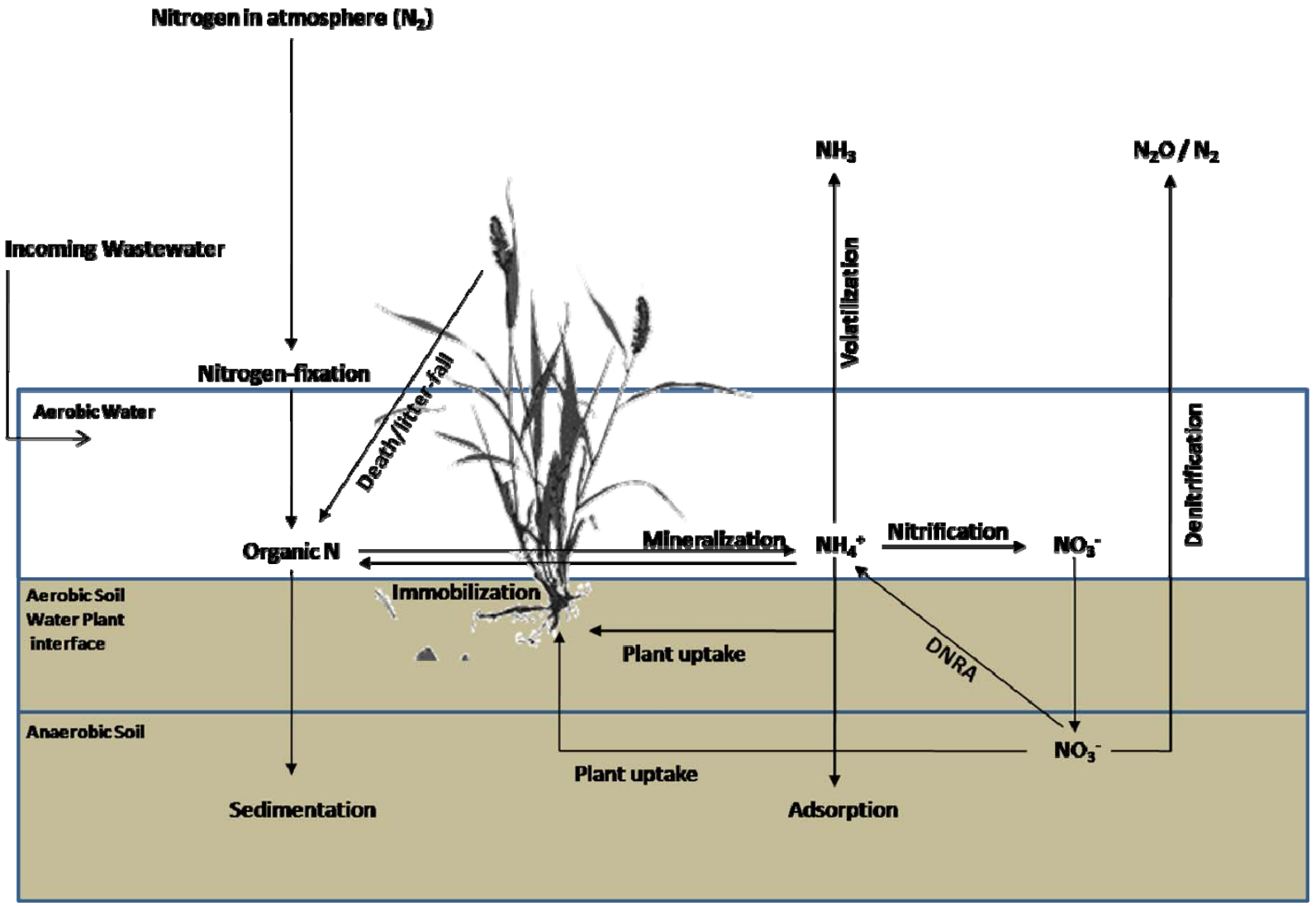

Figure 1-1. Diagram illustrating potential pathways for chemical transformations of nitrogen (N) in a wetland.

CHAPTER 2
MATERIALS AND METHODS

Site Description

The study was carried out at a constructed wetland at Monrovia Nursery, which is one of the largest (total area over 445 hectares) commercial nurseries in the United States, located in Cairo, Georgia. Cairo is located at 30°52′40″N, 84°12′32″W in Grady County, Georgia (Fig. 2-1), adjoining Leon and Gadsden counties of Florida. The annual production of the nursery ranges from 11 to 15 million plants (www.monrovia.com, October 2007).

Gravel beds are created outdoors on the ground and are overlaid with plastic sheets on which plants are grown in plastic containers ranging from 5 – 50 L capacity. In the catchment area for the wetlands, large (50 L) containerized ornamental trees and shrubs are grown in the sloped nursery beds, which are lined with plastic sheets. Fertilization of the nursery plants is done by two means; controlled release fertilizer (CRFs) mixed in the potting medium and fertigation (liquid fertilizers applied in irrigation water). Topdressing of CRFs is also done based on the needs of the plants. Overhead sprinkler or micro-irrigation is used approximately 3-5 times a day. While larger containers are irrigated via micro irrigation, runoff (Fig. 2-2) from these nursery beds is still significant due to the water application rates and sloped terrain. Runoff from the nursery beds and from stormwater is directed by lined or unlined waterways and drained into a flow control channel (FCC) which is 500 m in length (Fig. 2-3 and 2-4). Runoff eventually flows into a holding pond (HP) where it is held for recycling or for release into the wetlands. The FCC controls the movement of water and allows sedimentation to occur. Depending upon the holding capacity of the HP, the water from the FCC is either directed into the HP or diverted offsite through the stormwater retention basin. The HP is created in such a

way that it captures half an inch of rainfall and the excess rainfall is diverted either to the irrigation ponds for recycling or actively pumped into the wetlands.

A surface flow wetland was constructed at the nursery in 1997 to treat runoff effluents before releasing the excess water into a natural creek adjacent to the nursery property. This constructed wetland system is utilized to treat stormwater and runoff from three plant production areas (catchment area) totaling 48.5 hectares. The wetland was constructed by creating pits in a naturally depressed area of 3.8 hectares. A 12.5 cm think layer of bark chips was placed at the bottom of the pits to support the planting and also to serve as a source of carbon for the microbial activity in the constructed system. The wetland was constructed as several cells such as primary, secondary, and test cells (Fig. 2-5). The primary cells were designated as 1A and 2A; the secondary cells were designated as 1B and 2B; and the test cells were designated as 3,4,5,6, and 7. For this entire study, only one primary cell (1A) was used. There exists a difference in the size and depth of cells, and in the distribution and abundance of macrophytes between the primary and secondary cells. The area of the primary cells 1A and 2A is 1.17 hectares and 0.57 hectares respectively. The area of the secondary cells 1B and 2B is 0.69 hectares and 0.65 hectares respectively. The primary cells are 0.75 m deep and the secondary cells are 0.3m deep. The depth at the outflow (South) side of the primary cells is comparatively higher (1.2 m) than the inflow (North) side, which is 0.75 m deep. Each primary and secondary cell was divided into three sections by creating two earthen berms which extend to approximately ¾th of the width (north-south) of the cells. These earthen berms were created to maintain a linear water movement. The difference in vegetation of the primary and secondary cells is attributed to variation in planting selection and natural growth of plants. The macrophytes originally planted in the deeper primary cells were *Canna flaccida* Salisb. (Canna Lily), *Scirpus validus* Vahl.

(Giant Bulrush), *Pontederia cordata* L. (Pickerelweed), *Sagittaria latifolia* Willd. (Arrowhead), and *Panicum hemitomon* J.A Schultes. (Maiden cane). Currently the primary cells are dominated by *S. validus*, *Typha latifolia* L. (Cattail), *C. flaccida*, *Hydrocotyle umbellata* L. (Pennywort), *Lemna minor* L. (Duckweed), and *Wolffia* spp. Horkel ex Schleid. (Water meal). There are few open water areas in the primary cells and several floating islands of vegetation occur near the outflow (South) side of the cell. The macrophytes originally planted in the shallow cells were *C. flaccida*, *S. latifolia*, *P. hemitomon*, and *Juncus effusus* L. (Soft Rush). Currently the shallow secondary cells are predominantly covered by *T. latifolia*; and the other dominant species in the shallow cells are *S. latifolia*, *H. umbellata*, and *L. minor* (Duckweed).

When it is necessary to discharge water from the HP into the wetland, it is pumped through 6 cm inner diameter PVC pipes (Fig. 2-6) to the primary cells 1A and 2A, at 9 locations and 8 locations respectively. The water from each primary cell passes under a road [through 15 cm diameter PVC pipes; Fig. 2-7] and enters the secondary cell before being released into the grassed waterway that carries the treated water to the stilling pools. The water flow from primary to secondary cell and from secondary cell to the grassed waterway is based on gravity, and there is no external control to regulate the water flow. However, the water depth (and consequently the volume) of the primary cell can be controlled by adjusting the height of the inflow pipe of the secondary cell. The stilling pools enhance the settling of suspended sediments before discharging the treated water into the natural creek. The amount of water to be treated is influenced by the rate and frequency of rainfall and irrigation. Based on the amount of water available for treatment, four different 'run-times' (number of hours per day of operation of the pump) are utilized; 0, 12, 18, 24 hours per day. The most frequently used run-times are 18 and 24 hours per day. During very low rainfall and minimal irrigation period, the run-time of 12

hours or even 0 hours per day is used. The 12 and 18 hours per day run-times are achieved by running the pump for 2 hours and then turning it off for the next 2 hours, or 3- hours 'on' and 1-hour 'off' cycles, respectively, during a 24 hour period. The pump supplies water at an average of 2,271 liters per minute, which is distributed to all 17 inflow pipes of the primary cells. Hence the hydraulic loading rate of the wetland depends on the number of hours the pump is operational.

Field Methods

Water sampling (for determining the nutrient removal efficiency of the constructed wetlands). Water samples were collected from the study wetland cell at monthly intervals from April 2007 to August 2007. Three replicate water samples per section of the wetland cell were collected by taking grab samples in 125 ml plastic bottles (Fisher Scientific Co LLC, Suwannee, GA.) at 9 inflow points (one sample from each of the 9 pipes) and 3 outflow points (three replicate samples from each of the 3 pipes) as shown in Figure 2-8. In addition, three replicate samples were collected at a location where the flow control channel (FCC) releases water into the holding pond or into the stormwater retention basin. In summary, there were 9 inflow samples, 9 outflow samples and 3 FCC samples, totaling 21 samples for the water nutrient analysis. Samples were stored in a cooler filled with ice-packs, transported to the lab, and stored at 4°C until further processing. Water samples were analyzed for Total Kjeldal Nitrogen (TKN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP), and dissolved organic carbon (DOC).

Water sampling (for assessing the spatial effects on concentration of nutrients, physiochemical parameters and denitrification potential of the water column). Nutrient concentrations, physiochemical parameters, and denitrification potential were measured at select

sampling locations (Fig. 2-9). On the north side (the inflow side), one location was selected within each of the three sections of the primary cell from where the samples were collected. This sampling point was located approximately 3 m inward from the northern edge of the cell and approximately 3 m inward from the eastern edge. Two sets of three replicate water samples were collected at the surface and at 0.5 m depth of the water column at each of the three locations at monthly intervals between April 2007 and August 2007. One set of samples (three replications) was collected in 125 ml plastic bottles and analyzed for TKN, NH_4^+-N, NO_3^--N, TP, and DOC and the second set of samples (three replicates) was collected in 25 ml vials (Fisher Scientific, Pittsburgh, PA) and used to determine denitrification enzyme activity (DEA), to estimate denitrification potential in the samples. The surface water samples were collected as 'grab' samples in the undisturbed water column. To collect samples from the bottom of the water column, the sampling bottle was turned upside down and forced through the water column by hand. After reaching the bottom of the water column, the bottle was turned slowly to fill it and then rapidly brought straight up to the surface.

On the south side (outflow side) of the study cell, sampling points were selected approximately 2 m inward from the location of outflow pipe, to ensure minimum disturbance in the water column. Owing to its depth, the surface and bottom of the water column samples at the outflow side were collected by using a swing sampler. For the bottom of the water column samples, the bottle tied to the swing sampler was sent upside down through the water column. After reaching the bottom of the water column, the pole was turned slowly to fill the bottle and then rapidly brought straight up to the surface. Two sets of three replicate water samples were collected at the surface and bottom of the water column at each of the three locations at monthly intervals between April 2007 and August 2007. One set of samples (three replications) was

collected in 125 ml plastic bottles and analyzed for TKN, NH_4^+-N, NO_3^--N, TP, and DOC and the second set of samples (three replicates) was collected in 25 ml vials and analyzed for DEA. If the water was too turbid, suspended particles were allowed to settle by setting aside for a few minutes without any disturbance. Both surface and bottom of the water column samples were filtered by cheese cloth (Hermitage Inc, Camden, SC) to remove large debris, dead leaves, twigs, barks, and plants of *Wolfia* spp. In addition, three replicate water samples also were collected for denitrification potential analysis, at the point where the flow control channel (FCC) releases water into the holding pond or into the stormwater retention basin. There were 36 samples for nutrient analysis and 39 samples for denitrification potential analysis in total. Samples were stored in a cooler filled with ice-packs, transported to the lab, and stored at 4^0C until further processing.

In addition, pH, dissolved oxygen (DO), and temperature were measured every month during the study by using a handheld YSI 556 MPS (YSI Incorporated, Yellow Springs, OH) multi-probe system. The parameters were measured at both surface and bottom of the water column at inflow and outflow points on the days of water sample collection.

Plant sampling (for determining the denitrification potential of macrophyte rhizosphere soil). To assess the denitrification potential of macrophyte rhizosphere soil, two macrophytes were selected based on their abundance and distribution in the study cell, and based on the reported contribution of these macrophytes to nutrient removal in constructed wetlands. The plants selected for this study were T.latifolia and C. flaccida. Roots of three plants of each species were collected from each of the three sections at monthly intervals from plants growing on the north (inflow) side (Fig. 2-10). The sample plants were selected from where a group of the same species existed to avoid contamination with other species. The selected plants were

pulled out and the roots were severed and then placed in zip-lock bags, stored in a cooler filled with ice-packs, transported to the lab, and stored at 4^0C. There were 18 rhizosphere samples (three replicates of each species per section) in total.

Analytical Methods

Nutrient analyses. The day after sampling, part of all the water samples collected in 125 ml bottles was filtered through Whatman 0.45µm filter (Pall Corporation, Ann arbor, MI) and collected in two 25 ml vials for analysis of NH_4^+-N, NO_3^--N, and DOC. The remaining unfiltered part of each sample was used for analysis of TKN and TP. After filtering, all the filtered and unfiltered water samples (except the ones collected for analyzing denitrification potential), were acidified to a pH of 2 with one drop of ultra pure concentrated sulfuric acid (H_2SO_4) for every 20-25 ml of sample. The filtered and unfiltered portions of samples were stored in a refrigerator until further analysis. Samples were analyzed for respective nutrients within 28 days of sampling as recommended by the USEPA.

Total Kjeldal Nitrogen was measured by digesting the unfiltered, acidified samples by the Kjeldahl procedure (Method 351.2, USEPA, 1983). The water samples were digested with sulfuric acid (H_2SO_4) and a copper sulfate mixture ($CuSO_4$) to convert organic forms of nitrogen to ammonium. The digested samples were analyzed in $AQ2^+$ automated discrete analyzer (Seal Analytical, Mequon, WI) Method no: USEPA 111 A (Method 351.2, USEPA, 1983). Nitrate nitrogen was analyzed calorimetrically using the cadmium (Cd) reduction method on a rapid flow analyzer (Alpkem rapid flow Analyzer 300) or in the automated $AQ2^+$ discrete analyzer (Method 353.2, USEPA) or AQ2 Method no: USEPA 132 A (Method 353.2, USEPA 1983). During April, July, and August 2007 the filtered, acidified samples were analyzed for NO_3^--N on a rapid flow analyzer. In the month of May and June 2007, the discrete analyzer was used to

analyze NO_3^--N. Ammonium nitrogen was analyzed calorimetrically in filtered, acidified samples on $AQ2^+$ (Method no: USEPA 103 A (Method 350.1, USEPA, 1983). Total Nitrogen (TN) was calculated by summing up the TKN (organic nitrogen and $NH4^+$-N), and NO_3^- -N. To measure TP, unfiltered, acidified samples were digested by autoclaving and analyzed further by following the AQ2 Method no: USEPA 119 A (Method 365.2, USEPA, 1983). Dissolved organic carbon was analyzed in filtered, acidified samples on a Shimadzu TOC 5050a (USEPA 415.1). All the analysis were performed according the Quality Assurance/Quality Control requirements (a spike, repeat, continuing calibration standard, blank, Practical Quantitation Limit (PQL) to be run for every 20 samples) set by the University of Florida Wetland Biogeochemistry Lab.

Theoretical hydraulic residence time. The hydraulic residence time is a function of the ratio of wetland volume to water inflow rate. Because the study wetlands are operated for variable number of hours per day, the hydraulic loading rate (ratio of volumetric flow rate to wetland surface area/volume) under two predominantly occurring conditions, 18 and 24 hours pump operation per day, were factored into the calculations. The effect of vegetation (plant density) was also factored into the calculations while determining the theoretical residence time. The calculations were based on the assumption that the entire volume of water in the wetland is involved in the flow. The theoretical residence time was calculated by using the following formulae:

$\tau = V/Q$, in which
- τ = theoretical residence time in days;
- V = wetland water volume in m^3;
- Q = water flow rate in m^3/day.

The wetland volume (V) can be calculated using the formula:

$V = \varepsilon Ah$, in which

V = wetland water volume in m^3;
ε = wetland porosity in m^3/ m^3;
A = wetland area in m^2;
h = mean water depth in m.

Wetland porosity is a fraction of total wetland volume available through which water can flow (USEPA, 2000). It is the amount of wetland water volume not occupied by plants and expressed as a decimal (NRCS, 2002). Therefore, wetland porosity and plant density are inversely related; the lower the wetland porosity values, higher the plant density. As it is difficult to accurately measure wetland porosity in the field, highly variable porosity values were reported in the literature. For example, Reed et al. (1995) reported values ranging from 0.65 to 0.75 for fully vegetated wetlands, and for dense to less mature wetlands, respectively; Kadlec and Knight (1996) reported average wetland porosity values ranging from 0.95 to 1.0. According to USEPA (2000), it was suggested to use the wetland porosity value of 0.65 to 0.75 for fully vegetated wetlands, while considering design of constructed wetlands.

Denitrification potential (Water). Denitrification potential in water samples was determined by measuring the denitrification enzyme activity (DEA) within a week of sample collection (Tiedje, 1982; White and Reddy, 1999). Twenty ml of water sample (unfiltered) was taken into either 120 ml or 160 ml glass serum bottle. The bottles were capped and crimped ensuring proper seal. The bottles were purged with nitrogen gas (N_2) for approximately 5 minutes to achieve anaerobic conditions. The initial pressure of less than 20 psi was maintained. High grade acetylene (C_2H_2) gas was generated by adding N_2 purged water to a separate bottle filled with Calcium Carbide (CaC_2) rocks, which was already capped, crimped and subjected to N_2 purging to remove any oxygen. Nine ml of acetylene gas was injected into each sample bottle from which 9 ml headspace was removed before adding acetylene. Samples were then kept in a

shaker for an hour to ensure even distribution of C_2H_2 in the water samples. Denitrification Enzyme Assay (DEA) solution was prepared by adding nitrate (as KNO_3) and carbon (as $C_6H_{12}O_6$) at the rate of 404mg L^{-1} KNO_3 and 720 mg· L^{-1} $C_6H_{12}O_6$, respectively, along with 250 mg· L^{-1} chloramphenicol in distilled water. Chloramphenicol was added to inhibit the production of new denitrifying enzymes while the activity of previously existing denitrifying enzymes are measured. Eight ml of DEA solution was added to 20 ml sample. The 7 ml of head space of gas was collected by using a 10 ml syringe immediately after adding DEA to the samples and after taking the pressure readings. After that, the bottles were kept in an end-to-end shaker in a dark room at 25^{o}C. In the month of April 2007 the headspace gas samples were collected at 30, 60 120, 180 minutes. From May 2007 and to August 2007 the headspace gas samples were collected every 2 hours, up to 6 hours, as there was no gas production observed until 2 hours in the month of April. At the end of each pre-selected time period, 7 ml of gas (presumably nitrous oxide) was extracted by using a syringe and placed in 3-4ml capped, crimped, pre-evacuated glass serum bottles. The bottles with DEA solution and the original sample were then immediately returned to the shaker. The 7 ml sample of gas was stored at 25ºC until analyzed in a Gas Chromatograph (GC).

The concentration of Nitrous oxide (N_2O) was measured on a Shimadzu GC 14A (Shimadzu Scientific, Kyoto, Japan) by using a ^{63}Ni electron capture detector. Column temperature was 30^{o}C, detector temperature was 240 ^{o}C, and injector temperature was 120 ^{o}C. The carrier gas was a mix of 95% Argon (Ar) and 5% methane (CH_4). The gas samples were injected into GC and the concentration of N_2O was determined. Denitrification potential was determined by calculating the slope of the linear curve obtained when the gas concentrations were plotted over time.

Denitrification potential (Rhizosphere). The stored root samples were processed as early as possible after reaching the lab. The excessive and loosely adhering soil was removed by shaking the root system, and also by gently removing by hand. The soil very closely adhered to the roots was defined as 'rhizosphere soil' which was removed by washing the roots with autoclaved double distilled water. Thirty-five ml of water was used to remove the firmly attached soil by washing with the use of pipette. Twenty ml of the obtained soil slurry was used for the determination of denitrification potential. Ten ml of the rhizosphere solution was used to determine the moisture content of the soil. The moisture content was determined gravimetrically by drying 10 ml of the rhizosphere solution at 70^{o}C for 72 hours.

The denitrification potential of the rhizosphere soil was determined as described above. The volume of the glass serum bottle used in this experiment was 120 ml. Eight ml of DEA solution (720 mg· L^{-1} glucose, 404 mg· L^{-1} KNO^{3}, and 500 mg· L^{-1} chloroamphenicol) was added to 20 ml of samples. Gas samples were collected every 30, 60 120, 180 minutes. Rest of the procedure was as described for measuring denitrification potential in water samples.

Statistical Methods

Statistical analysis of the data was performed by using SAS 9.1 (SAS Institute Inc, Cary, NC). The dependent variables were subjected to analysis of variance (ANOVA) by using the GLM procedure along with Scheffe for all pair wise comparison, which indicate not only whether the means are different from each other, but also which means differ from which other means. Test of statistical significance was done at $\alpha = 0.05$. The physiochemical parameters of water viz., temperature and dissolved oxygen were correlated with denitrification potential by using CORR procedure to determine Pearson's correlation coefficient. The concentration of NO_3^--N was also correlated with denitrification potential by using the same procedure.

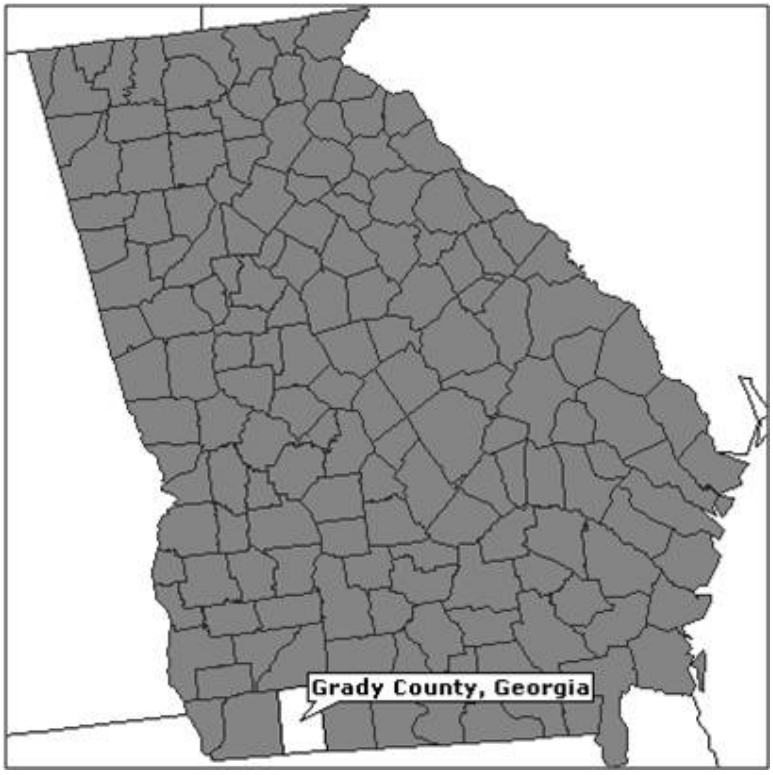

Figure 2-1. Map of the state of Georgia, United States, showing county boundaries. Grady County, in which Monrovia nursery is located, is indicated by a callout box. Source: http://www.epodunk.com/cgi-bin/genInfo.php?locIndex=7968, October 2007.

Figure 2-2. Runoff generated from the microirrigated nursery beds at Monrovia Nursery

Figure 2-3. Runoff generated from the nursery beds drains into the flow control channel

Figure 2-4. Runoff draining into the flow control channel

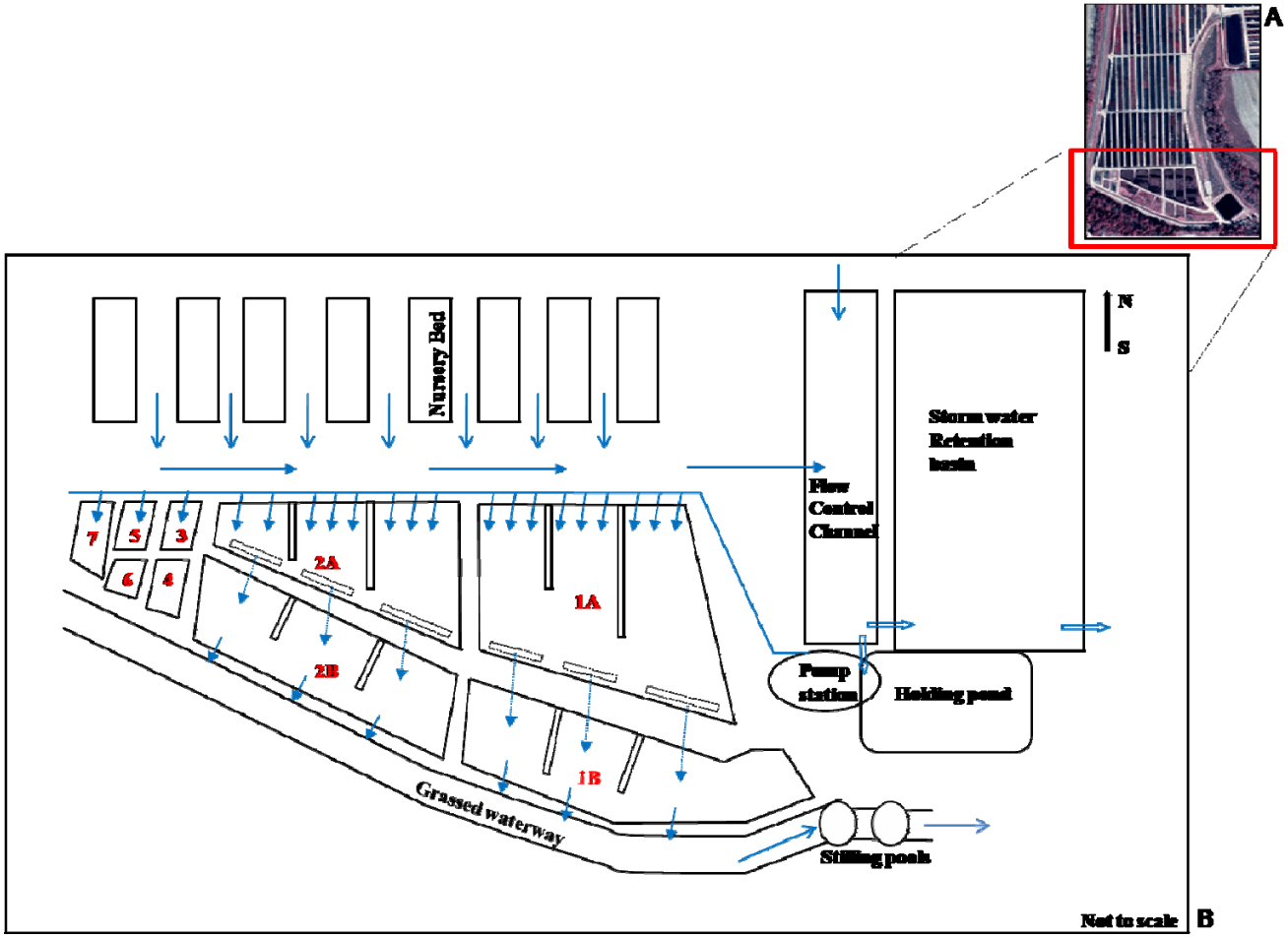

Figure 2-5. Overview of the Constructed wetlands at Monrovia Nursery, Cairo, GA. A) An aerial image (top right) of part of Monrovia Nursery, including the treatment wetlands, in Cairo, GA. B) A schematic overview of the constructed wetlands (bottom left) is also presented. The arrows indicate the direction of water movement through the wetlands. 1A and 2A, and 1B and 2B represent the primary and secondary cells, respectively.

Figure 2-6. Inflow pipes into the study cell

Figure 2-7. Outflow pipe of the study cell

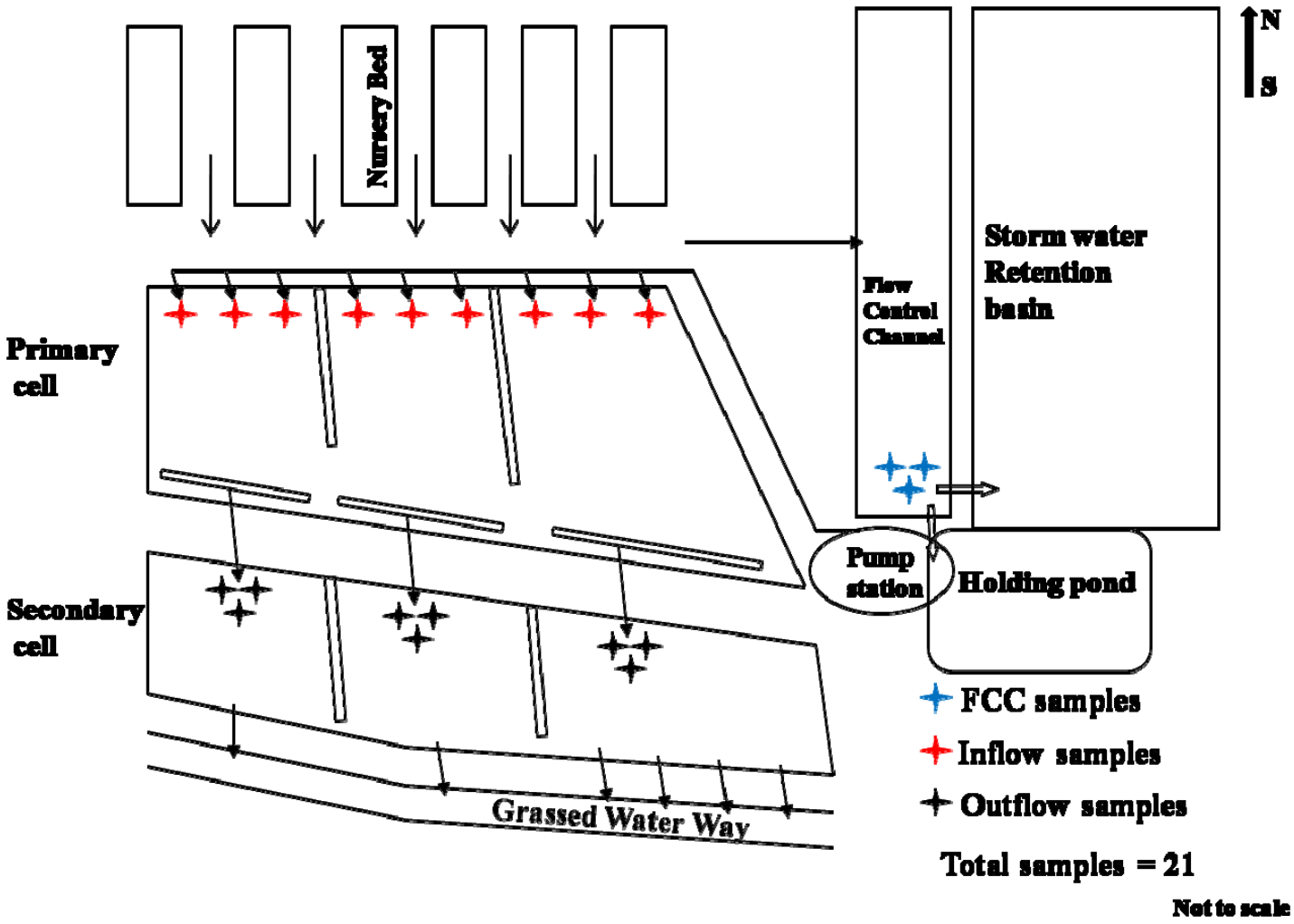

Figure 2-8. Location and number of water samples (indicated by stars) at inflow and outflow of the study cell to determine the nutrient removal efficiency of the constructed wetlands.

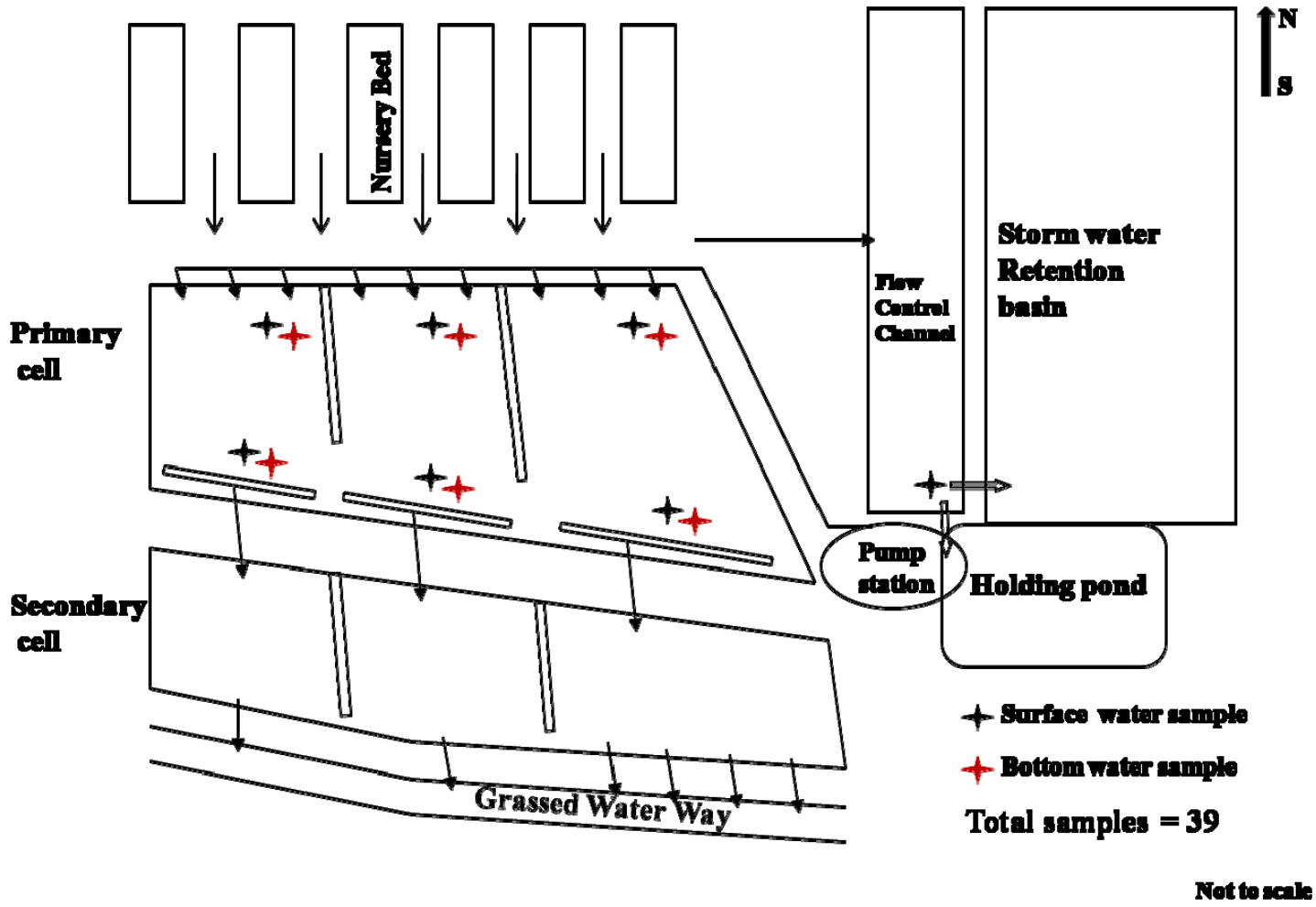

Figure 2-9. Locations of water samples (indicated by stars) in the study cell to assess the spatial variation in the concentration of nutrients and denitrification potential of the water column, between locations (inflow and outflow) and between depths (top and bottom) within locations over five months.

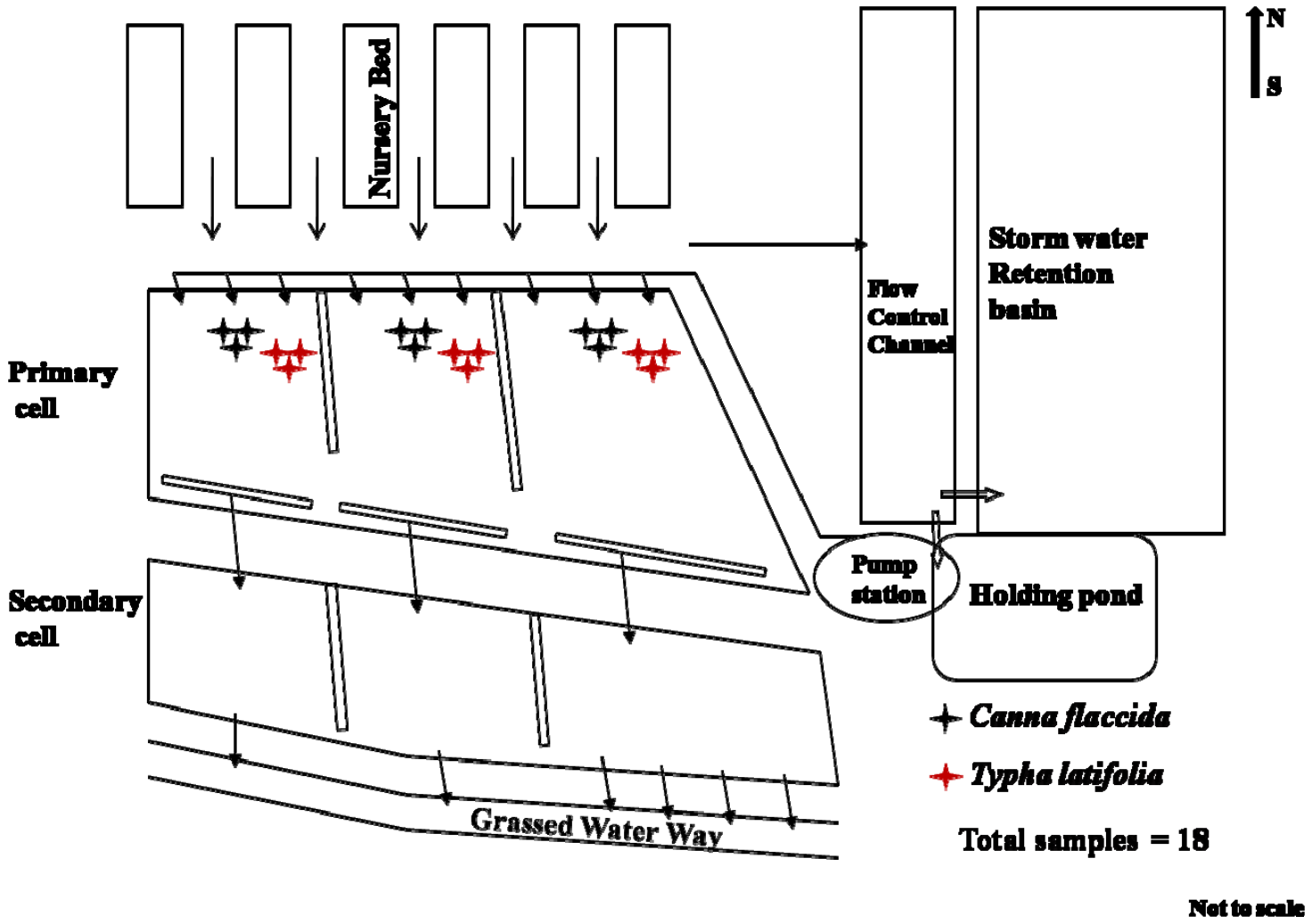

Figure 2-10. Illustration of the study cell showing the location and number (indicated by stars) of monthly plant root samples to assess the denitrification potential in the rhizosphere soil of *Canna flaccida* and *Typha latifolia*

CHAPTER 3
RESULTS

Removal efficiency of the constructed wetlands. The concentration of nutrients entering and leaving the constructed wetland was determined to evaluate the effectiveness of the study cell in removing nutrient contaminants over a period of five months from April to August 2007. The plant nursery runoff contained an average total nitrogen (TN) concentration of 34 mg L^{-1}during the study period. When assessing mean TN composition (Fig. 3-1), it was observed that the majority of Nitrogen (N) entering and leaving the wetland during the study period was in the form of nitrate nitrogen (NO_3^--N; 30 mg L^{-1}) followed by ammonium nitrogen (NH_4^+-N; 2 mg L^{-1}) and organic nitrogen (2 mg L^{-1}). Total phosphorus concentration was 1 mg L^{-1} and dissolved organic carbon was 10 mg L^{-1}. The nursery runoff entering the constructed wetland over the study period consisted of 87% of the TN in the form of NO_3^--N. The mean nutrient removal efficiency of the study cell, based on load-in and load-out rates, was observed to be 40% for TN, 40% for NO_3^- - N, 59% for NH_4^+-N, and 16% for TP (Table 3-1).

Nitrate Nitrogen (NO_3^--N). The concentration of NO_3^--N was influenced ($p<0.0001$) by an interaction among location of sample collection and month during which samples were collected (Fig. 3-2). The inflow NO_3^--N concentration gradually decreased from April (47 mg L^{-1}) to August (13 mg L^{-1}); similarly the outflow concentration also gradually decreased from April (42 mg L^{-1}) to August (3 mg L^{-1}). At flow control channel (FCC), NO_3^--N concentration did not show any trend over time, but the absolute values were highest in April (52 mg L^{-1}) and lowest in July (13 mg L^{-1}). The mean removal efficiency of NO_3^--N in the study cell was 40%, highest in August (77%) and lowest in April (12%). The removal efficiency was inversely related to the loading rate of NO_3^--N coming into the wetland; i.e., when the loading rate of NO_3^--N was higher (7 g m^{-2} day^{-1}; April), the removal efficiency was lower (12%) and when the loading rate was

lower (2 g m^{-2} day^{-1}; August), the removal efficiency was higher (77%). The concentration of NO_3^--N at FCC significantly differed from inflow concentration during the period of study, except for April. The mean inflow NO_3^--N concentration over the study period was 30 mg L^{-1}, which was significantly higher ($p<0.0001$) than the outflow concentration of NO_3^--N, averaging 18 mg L^{-1}.

Ammonium Nitrogen (NH_4^+-N). The nursery runoff that enters the constructed wetland over the study period consisted less than 1% of the TN in the form of NH_4^+-N. A significant interaction ($p < 0.0001$) was observed among location of sample collection and month during which samples were collected, for the concentration of NH_4^+-N (Fig. 3-3). . At all locations (inflow, outflow, and FCC), the NH_4^+-N concentration did not show any particular trend over months, but the mean values were always higher at inflow and lower at outflow for each month of this study. The average removal efficiency of NH_4^+-N was 59% and there was no trend observed over months. There were significant differences in the concentration of NH_4^+-N between FCC and inflow concentration over months, which indicate that the NH_4^+-N removal in the holding pond was noticeable. The average concentration of the NH_4^+-N coming into the wetland over the study period was 2.1 mg L^{-1} which was higher ($p<0.0001$) than the concentration of NH_4^+-N leaving the system, averaging 0.9 mg L^{-1}.

Total Nitrogen (TN). The TN was computed by summing the values of Total Kjeldal Nitrogen (TKN) and NO_3^--N. The trend was similar to that of NO_3^--N. The concentration of the TN was influenced ($p<0.0001$) by an interaction among location of sample collection and month during which samples were collected (Fig. 3-4). The inflow concentration of TN gradually decreased from April (60 mg L^{-1}) to August (15 mg L^{-1}); similarly the concentration of TN at outflow was also gradually decreased from April (49 mg L^{-1}) to August (4 mg L^{-1}). At FCC, TN

concentration was highest in April (70 mg L^{-1}) and lowest in July (15 mg L^{-1}), but did not appear to consistently increase or decrease over this period of time. Overall, the average removal efficiency of TN was 40% which gradually increased from April 2007 (18%) to August 2007 (73%). It was observed that the removal efficiency was inversely related to the loading rate of TN into the wetland. The mean concentration of TN over five months at inflow (34 mg L^{-1}) was significantly higher than the concentration of total nitrogen at outflow (20 mg L^{-1}) of the study cell.

Total Phosphorus (TP). The concentration of TP was influenced ($p<0.0001$) by location of sample collection and month during which samples were collected. The concentration of TP at all three locations (inflow, outflow, and FCC) did not follow any particular trend between April and August 2007 (Fig 3-5). The inflow concentration of TP over months ranged from 1.7 mg L^{-1} (April) to 0.8 mg L^{-1} (August); and the concentration of TP at outflow ranged from 1.4 mg L^{-1} (April) to 0.8 mg L^{-1} (August). Although the interaction among location of sample collection and month showed significant influence, most of the means were statistically similar. The removal efficiency of TP ranged from 0-32%, which varied over months. In July 2007, the removal efficiency was negative, which indicates that there might be possible internal loading of phosphorus in the system. There was no significant difference in the concentration of TP between FCC and inflow of the wetland. The mean concentration over five months, of TP coming into the wetland was 1.3 mg L^{-1}, which was higher ($p<0.0001$) than the concentration of TP leaving the system, averaging 1.1 mg L^{-1}.

Dissolved Organic Carbon (DOC). The concentration of DOC was influenced ($p<0.0001$) by an interaction among location of sample collection and month during which samples were collected (Fig 3-6). The DOC concentration at inflow ranged from 10 mg L^{-1}

(June) to 12 mg L^{-1} (August), and at outflow it ranged from 10 mg L^{-1} (July) to 11 mg L^{-1} (May) Most of the means were statistically similar, although the overall model showed significant interaction among locations and months. The five month average concentrations of DOC at FCC and inflow of the study cell were similar. The mean concentration of DOC present in the inflow water was 11 mg L^{-1} which was higher ($p<0.001$) than the concentration of DOC leaving the system, averaging 10 mg L^{-1} during the study period.

TN: TP ratio. The computed TN: TP ratio was affected ($p<0.0001$) by an interaction among location of sample collection and months during which samples were collected. At inflow, the ratio was highest in the month of April (36:1) and lowest in July (17:1). The ratio at outflow was also highest in April (35:1), but it was lowest in August (5:1). At FCC, the ratio ranged from 12:1(July) to 33:1(August). The five month mean TN: TP ratio, at inflow and outflow, was 25:1 and 18:1, respectively.

DOC: NO_3^--N ratio. The computed DOC: NO_3^--N ratio was affected ($p<0.0001$) by an interaction among location of sample collection and months during which samples were collected. At both inflow and outflow, there was gradual increase in the DOC: NO_3^--N ratio over five months. At inflow, the ratio gradually increased from April (0.2:1) to August (0.9:1); similarly the ratio at outflow also gradually increased from April (0.2:1) to August (3.3:1). At FCC, the ratio ranged from 0.3:1(April and May) to 0.8:1(July), with values not showing any trend over the period of the study. The five month mean DOC: NO_3^--N ratios at inflow and outflow were 0.5:1 and 1.3:1, respectively.

Relationship between nutrient loading and removal rates. The correlation between loading and removal rate (g m^{-2} day^{-1}) of NO_3^- - N, NH_4^+-N and TP are presented in Figure 3-7 to 3-9. The Pearson's correlation coefficient (r) value of 0.19 ($p<0.21$) was obtained for the

relationship between loading and removal rate of NO_3^- - N. The NH_4^+-N and TP exhibited a linear relationship with Pearson's correlation coefficient (r) value of 0.75 ($p<0.0001$) and 0.60 ($p<0.0001$), respectively.

Determination of theoretical hydraulic residence time. The effect of hydraulic loading rate and plant density on hydraulic residence time was computed by utilizing a model and the results are shown in Figure 3-10. The residence time was calculated for two different loading rates with three different plant density conditions, which provide three different wetland porosity values. Hydraulic loading rate was inversely related to residence time; i.e., when the loading rate increased from 1300 to 1734 m^3day^{-1}, the theoretical residence time decreased by a day for all three wetland porosity values. As the wetland porosity value increased from 0.75 to 0.95, the theoretical residence time increased at least by one day in this model. There was no difference in the residence time when the wetland porosity value increased from 0.95 to 1.0 at both hydraulic loading rates.

Spatial effects on nutrients, physiochemical parameters and denitrification potential of the water column:

Nitrate Nitrogen (NO_3^--N). The concentration of NO_3^--N was not influenced ($p=0.0812$) by an interaction among the treatments (location, depth, and months), presented in table 3-2. All two-way interactions, depth*month, location*month, and location*depth significantly affected the concentration of NO_3^--N at $\alpha=0.05$ level. The interaction among the location of sample collection (inflow and outflow) and the months during which samples were collected, showed that the concentration of NO_3^--N changed from inflow to outflow except in April. In April, the inflow concentration (44 mg L^{-1}) of NO_3^--N was similar to that at outflow (42 mg L^{-1}), but it was significantly higher than other means. There was no trend observed

between April and August, both in inflow and outflow concentration of NO_3^--N, but the concentration during each month was higher at inflow than at outflow. The interaction among location of sample collection (inflow and outflow) and depth within location (surface and bottom of the water column) showed that surface (24 mg L^{-1}) of the water column at inflow had higher mean concentration of NO_3^--N than at the lower depth (21 mg L^{-1}) at inflow and the surface (18 mg L^{-1}) of outflow (Fig. 3-11). On the outflow side, the mean concentration of NO_3^--N over five months was higher (18 mg L^{-1}) at the surface than the lower depth of the water column (16 mg L^{-1}). The five month mean concentration of NO_3^--N was always higher at surface water column as compared to lower depth of the water column.

Ammonium Nitrogen (NH_4^+-N). The mean concentration of NH_4^+-N was influenced (p=0.0001) by an interaction among location, depth, and months (Table. 3-2). Most of the means were similar, however. The distribution of NH_4^+-N within the wetland did not vary. The concentration of NH_4^+-N was also influenced by an interaction among location*month, and by depth*month. Although both depth*month, and location*month interactions yielded some significant means, there was no clear trend over time observed in the concentration of NH_4^+-N. At inflow, the concentration of NH_4^+-N ranged from 3.1 mg L^{-1} (April) to 0.2 mg L^{-1} (May). At outflow the NH_4^+-N concentration ranged from 0.1 mg L^{-1} (May) to 1.8 mg L^{-1} (June).

Total Phosphorus (TP). The concentration of TP was influenced (p=0.0009) by an interaction among location of sample collection, depth within locations, and month (Table 3-2), however most of the means were similar. The absolute TP concentration at inflow and outflow was higher at lower depth of the water column than that of the surface of the water column between April to August. In addition to three-way interaction, location of sample collection*depth was observed to highly affect TP concentration. At the inflow, the surface (2

mg L^{-1}) TP concentration was lower than the TP in the bottom (3 mg L^{-1}) of the water column. In contrast, the concentrations of TP at the two depths were similar at outflow. The other two-way interactions between location*month, and depth * month did not show any particular trend in the concentration of TP during the period of this study.

Dissolved Organic Carbon (DOC). The concentration of DOC was affected (p=0.0008) by an interaction among location, depth, and month (Table 3-2). At inflow and outflow, the surface and lower depth of the water column did not show difference in the concentration of DOC over the five months of this study. There was no trend observed for the DOC concentrations during this study for both locations and depth within location. Of all the two-way interactions only location* depth showed significant differences between treatment means. The surface of the water column at inflow had higher (11 mg L^{-1}) concentration of DOC as compared to surface (10 mg L^{-1}) of the water column at outflow. Similarly the DOC concentration was higher (12 mg L^{-1}) at inflow and lower at outflow (10 mg L^{-1}) for bottom of the water column. The five month mean concentration of DOC was similar at two depths both at inflow and outflow of the wetland.

DOC: NO_3^--N ratio. The DOC: NO_3^--N ratio was influenced (p=0.0009) by a three-way interaction among location, depth, and months. At inflow, the DOC: NO_3^--N ratio at the lower depth of the water column was higher as compared to the surface water column between April to August. Similar trend of higher DOC: NO_3^--N ratio at the lower depth was observed at the outflow of the study cell, except for April. However, there was no temporal pattern observed during this period both at inflow and outflow of the study cell. All two-way interactions, depth*month, location*month, and location*depth, significantly influenced the ratio at α=0.05 level. The mean DOC: NO_3^--N ratio over five months at the inflow and outflow was 0.9:1 and

1.5:1, respectively. The five month mean DOC: NO_3^--N ratio at the surface and bottom of the water column were 1.0:1 and 1.3: 1, respectively.

Temperature. The temperature of the water column was affected (p=0.0141) by an interaction among locations (inflow and outflow), depth within location (surface and bottom), and months (April to August 2007). Although the overall model showed significant difference, the mean comparisons showed similar values for the bottom and surface of the water column temperature both at inflow and outflow during the period of this study (Table. 3-3). For bottom of the water column on the inflow side, the effect of months on temperature was apparent as it gradually increased from May (22^{0}C) to August (30^{0}C). The temperature at lower depth of the water column at outflow was also significantly affected by month of sampling. Surface water temperature on the inflow side was lowest during May (23^{0}C) and highest (30^{0}C) in August. Similar trend over time was observed at the surface of the water column on the outflow side of the study cell. There was significant difference between bottom (22^{0}C) and surface (25^{0}C) water column temperature at outflow during the month of May, but the temperature at surface and lower depth of the water column was similar during other months. The five month mean temperature (averaged over surface and lower depth values) of the wetland at inflow (27^{0}C) was higher than the outflow (26^{0}C). The five month mean temperature at the surface of the water column (averaged over inflow and outflow locations) was higher (26^{0}C) than the bottom (27^{0}C) of the water column.

Dissolved Oxygen. The dissolved oxygen (DO) concentration in the water column was not affected by any interaction effect (Table 3-3) between the treatments (locations, depth and months). The main effects of location of sample collection, depth of the water column and months showed significant difference at $\alpha = 0.05$ level (Fig. 3-12). The dissolved oxygen

concentration of the inflow (6 mg L^{-1}) was significantly (p=0.0005) lower when compared to that of outflow (11 mg L^{-1}). The DO was observed to significantly decrease from surface (11 mg L^{-1}) to bottom (6 mg L^{-1}) of the water column. Although the overall analysis showed significant difference between months, only the value for May (12 mg L^{-1}) was significantly different from rest of the months.

pH. The pH was not affected by interactions between locations, depth and months (Table 3-3), except for interaction among location and months (Fig. 3-13), and because of the main effect of months. At inflow, the pH in August (4) was lower than in May (6), June (7) or July (6). In August, the inflow pH (4) was lower than outflow pH (6). The other interactive means were similar. When compared over months, mean pH in June was higher (7) than the values obtained for July (6) and August (5). The mean pH of this system was 6 during the period of this study.

Denitrification potential of water. The concentration of nitrous oxide nitrogen (N_2O-N) produced per liter of water per hour (mg N_2O-N L^{-1} hr^{-1}) was calculated and it was observed that the N_2O-N production was either negligible (0.01 to 0.03 mg N_2O-N L^{-1} hr^{-1}) or undetectable, as was the case in most of the samples. The data, thus, are neither presented graphically, nor in a tabular form.

Denitrification potential of macrophyte rhizosphere soil. The denitrification potential of the rhizosphere soil was influenced (p=0.0419) by an interaction among macrophyte species, sections, and months (Table. 3-4). The significant differences for the denitrification potential were observed between very few means, however. The rhizosphere soil of *Typha latifolia* produced significantly higher denitrification potential (30 mg N_2O-N kg^{-1} hr^{-1}) in the western section of the cell during May, which was higher than the values obtained from the rest of the

means. The two-way interaction among month*section and plant*month occurred, however most of the means were statistically similar. During May 2007, the denitrification potential of *T. latifolia* was 12 mg N_2O-N kg^{-1} hr^{-1}. Similarly the denitrification potential in the western section (17 mg N_2O-N kg^{-1} hr^{-1}) was found to be significantly different from rest of the treatments during the month of May 2007. Denitrification potential was influenced ($p = 0.01$) by the main effect of month; however, there was no trend between April and August 2007. The mean denitrification potential was 8, 2, 1, 3 mg N_2O-N kg^{-1} hr^{-1}, during May, June, July, and August 2007, respectively. Only the denitrification potential during May differed from other months. The five month mean denitrification potential for *Canna flaccida* and *Typha latifolia* were 3 mg and 4 mg N_2O-N kg^{-1} hr^{-1}, respectively.

Table 3-1. Mean load-in, load-out, removal rate (g m^{-2} day^{-1}) of nutrients and percentage nutrient removal efficiency of the constructed wetland over five months (April and August 2007). Standard deviation values in are presented in parentheses.

	Total Nitrogen (TN) (g m^{-2} day^{-1})	Nitrate Nitrogen (NO_3^-) (g m^{-2} day^{-1})	Ammonium Nitrogen (NH_4^+) (g m^{-2} day^{-1})	Total Phosphorus (TP) (g m^{-2} day^{-1})
Load-in rate	5.07 (2.64)	4.43 (2.21)	0.32 (0.21)	0.19 (0.05)
Load-out rate	3.07 (2.50)	2.68 (2.18)	0.13 (0.14)	0.16 (0.04)
Removal rate	1.99 (0.15)	1.75 (0.75)	0.19 (0.11)	0.03 (0.03)
% Removal	40	40	59	16

Table 3-2. Mean concentrations of nutrients (mg L^{-1}) as affected by location of sample collection, depth within location, and months (April and August 2007) in the constructed wetland receiving nursery runoff (n=18). Capital letters indicate statistical difference between depths (top and bottom) at inlet and outlet over five months; lower-case letters indicate statistical difference between locations (inlet and outlet) at top and bottom depths over five months; asterisks(*, **) indicate statistical difference between locations at top and bottom for every month. Means followed by the same letter and same number of asterisks are statistically similar.

Location	Depth	Month	NO_3^--N	mg L^{-1}	NH_4^--N	mg L^{-1}	TP	mg L^{-1}	DOC	mg L^{-1}
Inlet	Top	April	46	A a *	4.0	A a *	1.8	A a *	11	A a *
		May	42	B a *	0.2	BC a *	2.0	A a *	13	B a *
		June	8	D a *	2.3	AB a *	1.4	A a *	10	A a *
		July	15	C a *	0.2	B a *	1.3	A a *	11	A a *
		Aug	11	CD a *	1.3	ABC a *	0.9	A a *	11	A a *
	Bottom	April	42	A a *	2.2	AB a *	2.1	A a *	11	AB a *
		May	36	B b *	0.2	B a *	2.4	A a *	12	AB a *
		June	7	D a *	2.0	AB a *	3.5	A b *	10	B a *
		July	11	C a *	0.9	BC a *	2.3	A a *	11	AB a
		Aug	7	CD b *	3.6	A a *	3.0	A b *	13	A a *
Outlet	Top	April	43	A a *	1.0	A a *	1.6	A a *	11	A a *
		May	32	B a **	0.1	A a *	1.2	A a *	11	A a *
		June	4	C a **	1.7	A a *	0.9	A a *	9	A a *
		July	7	C a **	0.1	A a *	1.2	A a *	9	A a *
		Aug	4	C a **	0.1	A a *	0.7	A a *	10	A a *
	Bottom	April	42	A a *	2.3	A a *	1.5	A a **	10	A a *
		May	26	B a **	0.1	A a *	1.3	A a *	11	A a *
		June	4	C a *	1.8	A a *	0.9	A a *	10	A a *
		July	5	C a **	0.1	A a *	1.5	A a **	9	A a *
		Aug	3	C b **	0.1	A a **	1.0	A a *	10	A a **

Table 3-3. Physiochemical parameters (temperature, dissolved oxygen, pH) as affected by location of sample collection, depth within location, and months (April and August 2007) in the constructed wetland receiving nursery runoff (n=3). Capital letters indicate statistical difference between depths (top and bottom) at inlet and outlet over five months; lower-case letters indicate statistical difference between locations (inlet and outlet) at top and bottom depths over five months. Means followed by the same letter are statistically similar.

Location	Depth	Month	Temperature ˚C	Dissolved oxygen (mg L^{-1})	pH
Inlet	Top	May	23 A a	13 A a	6.3 A a
		June	28 A a	7 A a	7.2 A a
		July	28 A a	7 A a	7.0 A a
		Aug	30 A a	6 A a	4.4 A a
	Bottom	May	22 A a	4 A a	6.2 A a
		June	27 A a	5 A a	7.2 A a
		July	27 A a	3 A a	5.4 A a
		Aug	30 A a	2 A a	3.7 A a
Outlet	Top	May	25 A a	25 A a	5.5 A a
		June	27 A a	15 A a	6.5 A a
		July	27 A a	11 A a	5.8 A a
		Aug	29 A a	12 A a	6.9 A a
	Bottom	May	22 B a	15 A a	6.4 A a
		June	25 A a	10 A a	6.6 A a
		July	26 A a	5 A a	5.0 A a
		Aug	28 A a	4 A a	6.0 A a

Table 3-4. Denitrification potential (mg N_2O-N kg^{-1} hr^{-1}) as affected by macrophyte rhizosphere soil in three sections (east, middle, and west) between May 2007 and August 2007 in the constructed wetland receiving nursery runoff. (n =3). Capital letter indicates the statistical difference between plant species within each section and month. Means followed by the same letter are statistically similar.

Plant species	**Month**	**Section**	**Denitrification potential**	
			mg kg^{-1} hr^{-1}	
Canna flaccida	May	East	1.9	A
		Middle	2.4	A
		West	4.1	A
	June	East	2.0	A
		Middle	2.7	A
		West	3.4	A
	July	East	1.0	A
		Middle	0.5	A
		West	3.1	A
	August	East	2.2	A
		Middle	3.9	A
		West	3.0	A
Typha latifolia	May	East	1.5	A
		Middle	6.1	A
		West	29.7	A
	June	East	2.1	A
		Middle	0.2	A
		West	0.1	A
	July	East	0.6	A
		Middle	0.3	A
		West	0.1	A
	August	East	6.1	A
		Middle	0.3	A
		West	2.3	A

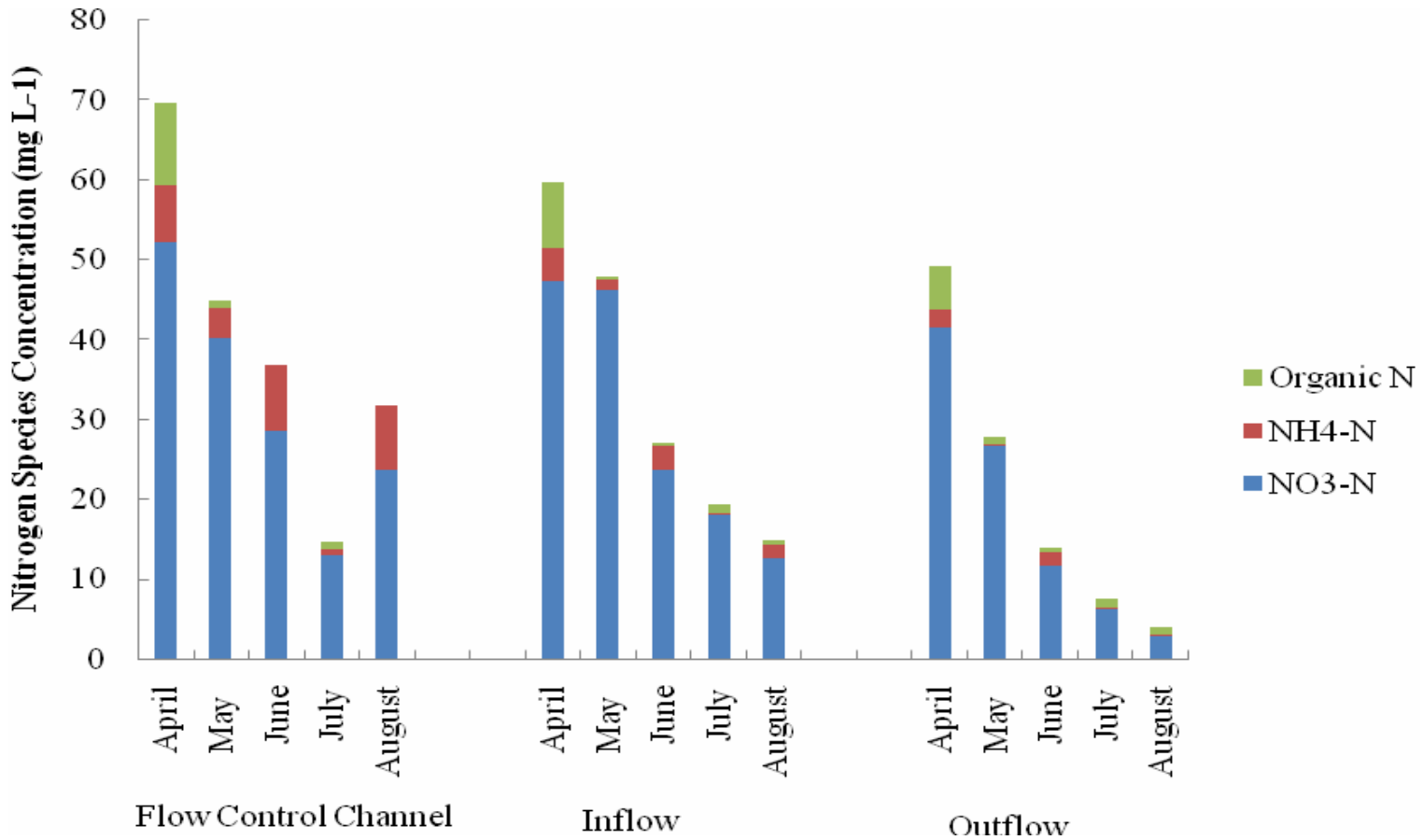

Figure 3-1. Mean concentration (mg L^{-1}) of total nitrogen (TN) and composition of TN [nitrate (NO_3^- - N), ammonium (NH_4^+-N) and organic nitrogen concentration, mg L^{-1}] between April to August of 2007 in the water samples obtained from the flow control channel (n=3), inlet (n=9), and outlet (n=9) pipes of a constructed wetland receiving nursery runoff.

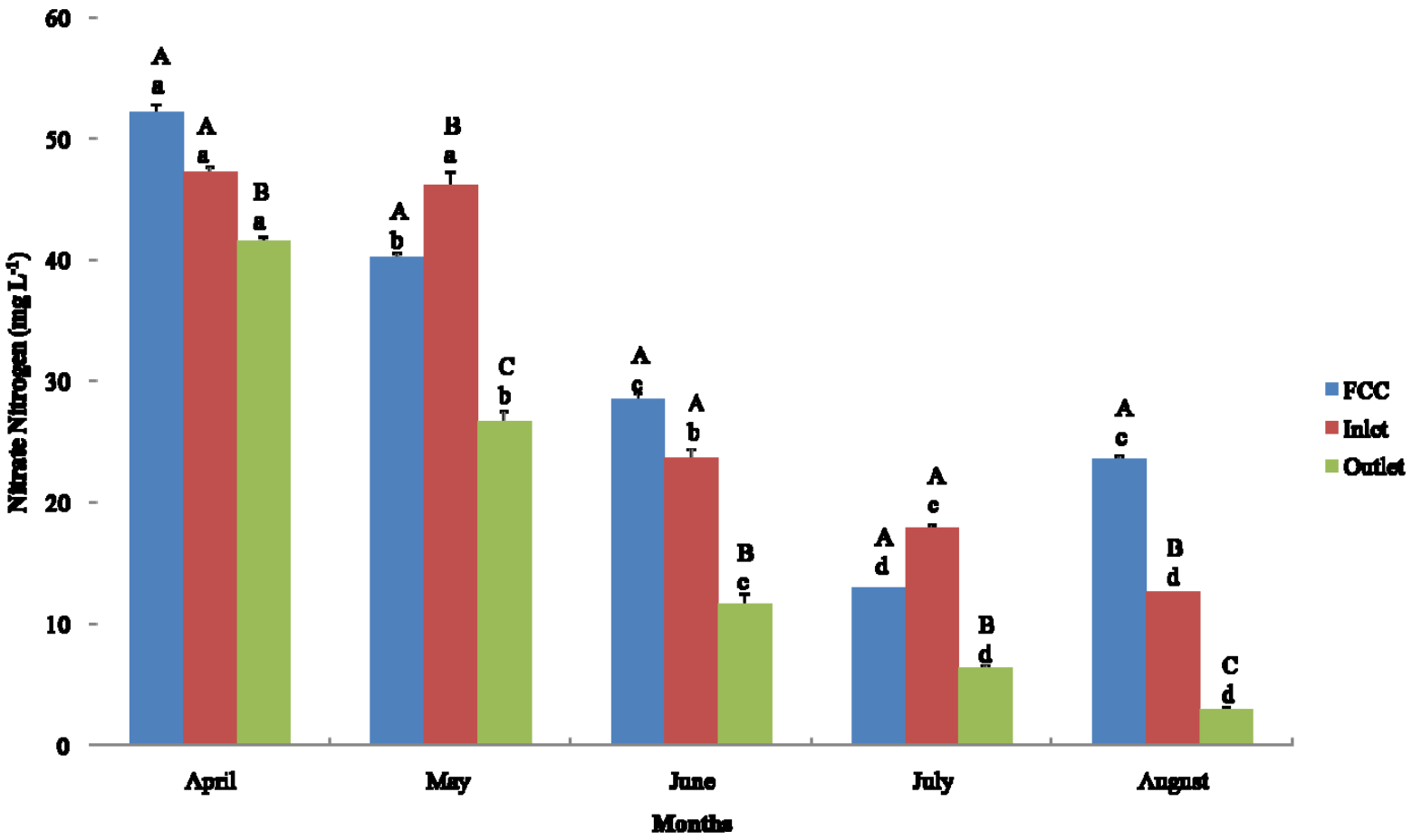

Figure 3-2. Mean concentration (mg L^{-1}) of nitrate nitrogen (NO_3^--N) between April 2007 and August 2007 in the flow control channel (n=3), inlet (n=9) and the outlet (n=9) of the study cell in the constructed wetland receiving nursery runoff. Error bars indicate plus standard error. Capital letter indicates statistical difference between locations within each month (May to August); lower-case letters indicate statistical difference between months at each location (FCC, inlet and outlet). Means followed by the same letter are statistically similar.

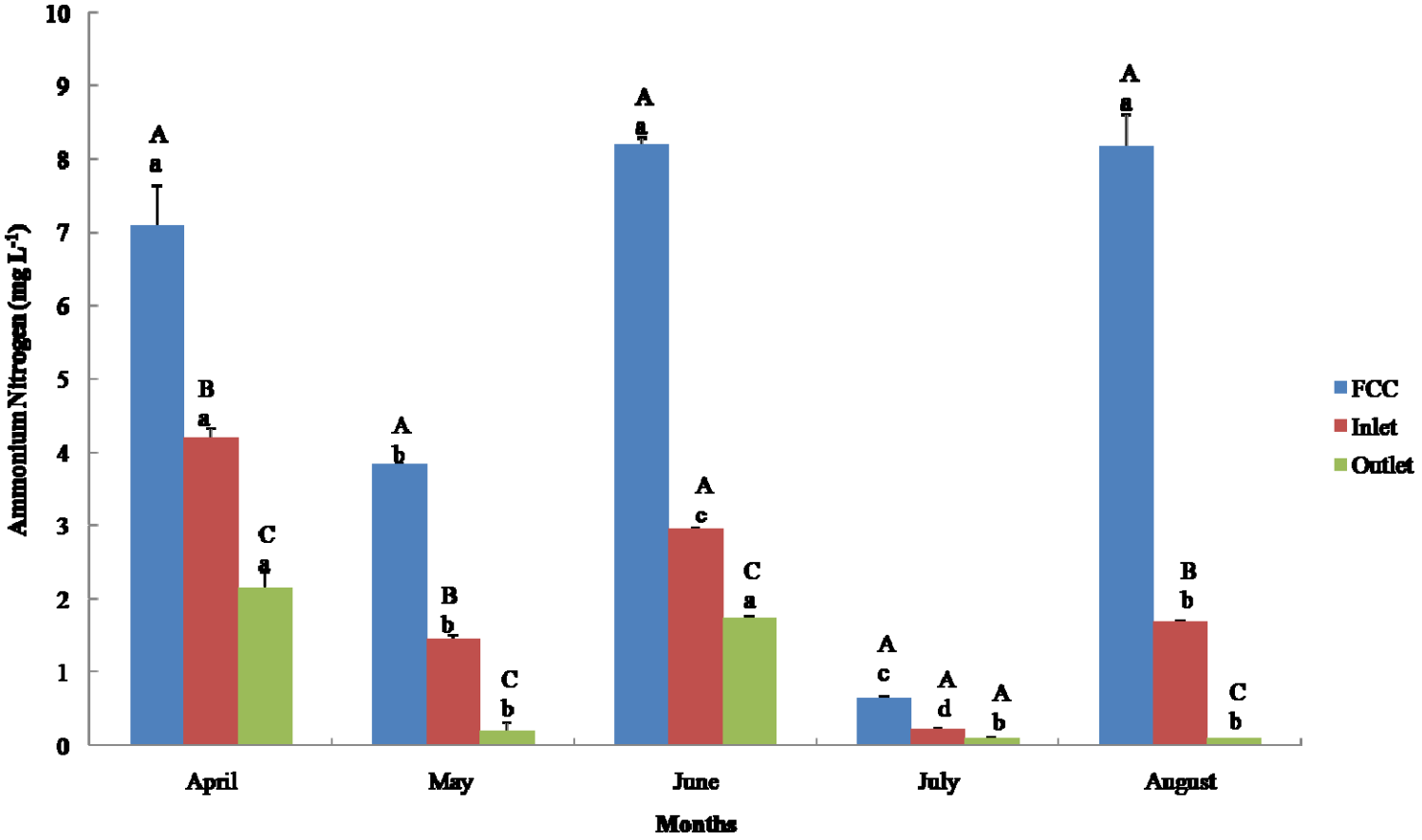

Figure 3-3. Mean concentration (mg L^{-1}) of ammonium nitrogen ($NH4^{+}$ - N) between April 2007 and August 2007 in the flow control channel (n=3), inlet (n=9) and the outlet (n=9) of the study cell of a constructed wetland receiving nursery runoff. Error bars indicate plus standard error. Capital letter indicates statistical difference between locations within each month (May to August); lower-case letters indicate statistical difference between months at each location (FCC, inlet and outlet). Means followed by the same letter are statistically similar.

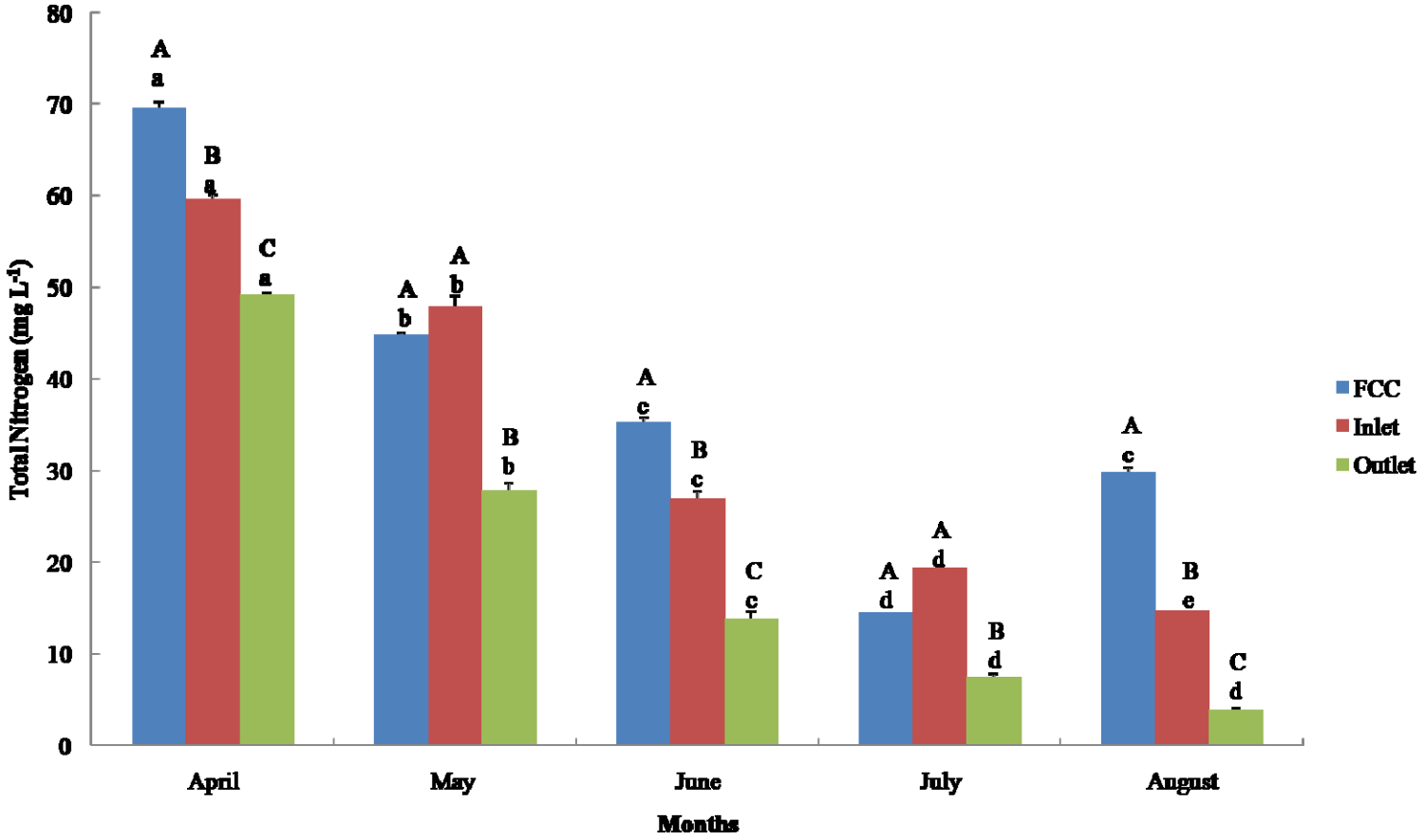

Figure 3-4. Mean concentration (mg L^{-1}) of total nitrogen (TN) between April 2007 and August 2007 in the flow control channel (n=3), inlet (n=9) and the outlet (n=9) of the study cell of a constructed wetland receiving nursery runoff. Error bars indicate plus standard error. Capital letter indicates statistical difference between locations within each month (May to August); lower-case letters indicate statistical difference between months at each location (FCC, inlet and outlet). Means followed by the same letter are statistically similar.

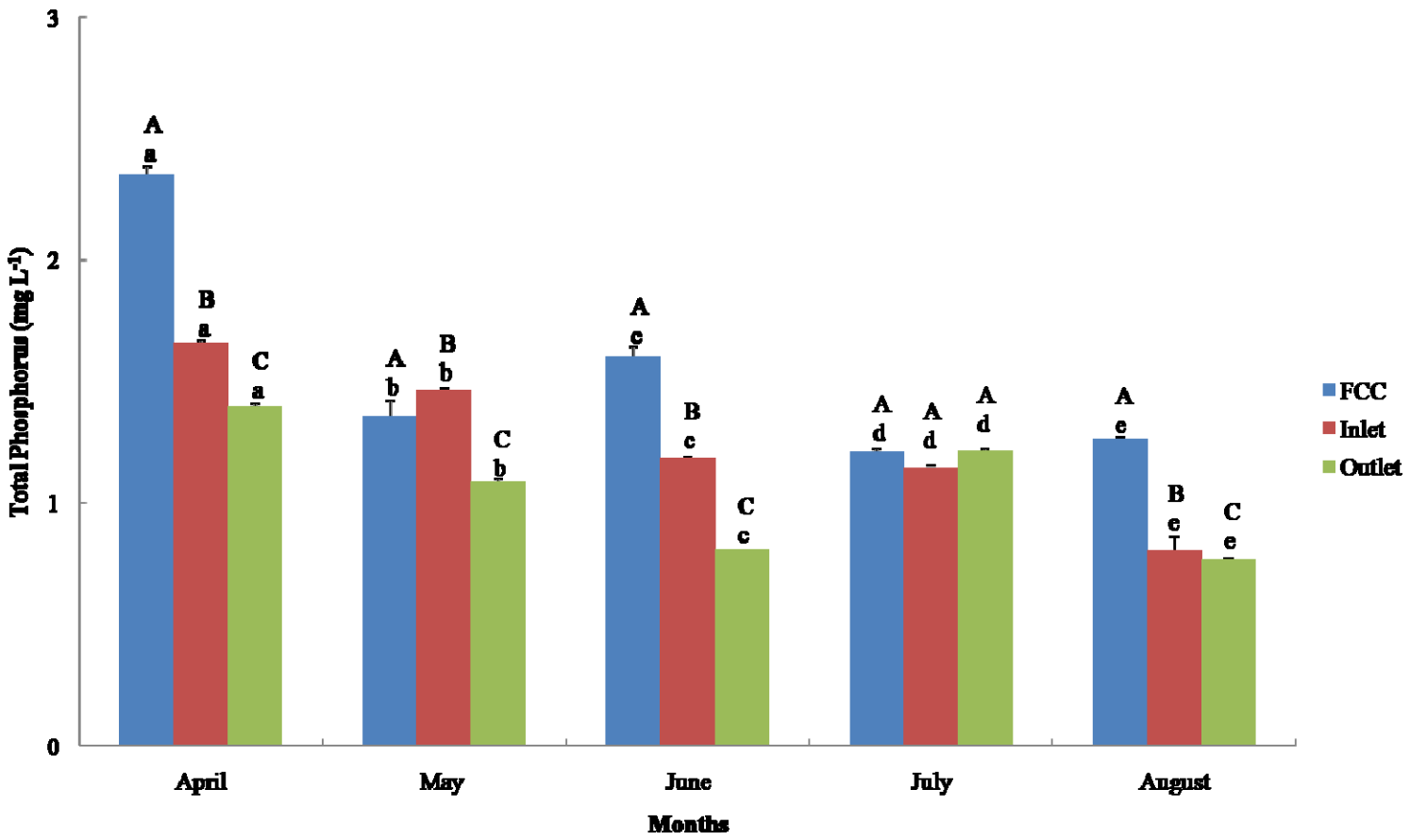

Figure 3-5. Mean concentration (mg L^{-1}) of total phosphorus (TP) and Dissolved organic carbon between April 2007 and August 2007 in the flow control channel (n=3), inlet (n=9) and the outlet (n=9) of the study cell of a constructed wetland receiving nursery runoff. Error bars indicate plus standard error. Capital letter indicates statistical difference between locations within each month (May to August); lower-case letters indicate statistical difference between months at each location (FCC, inlet and outlet). Means followed by the same letter are statistically similar.

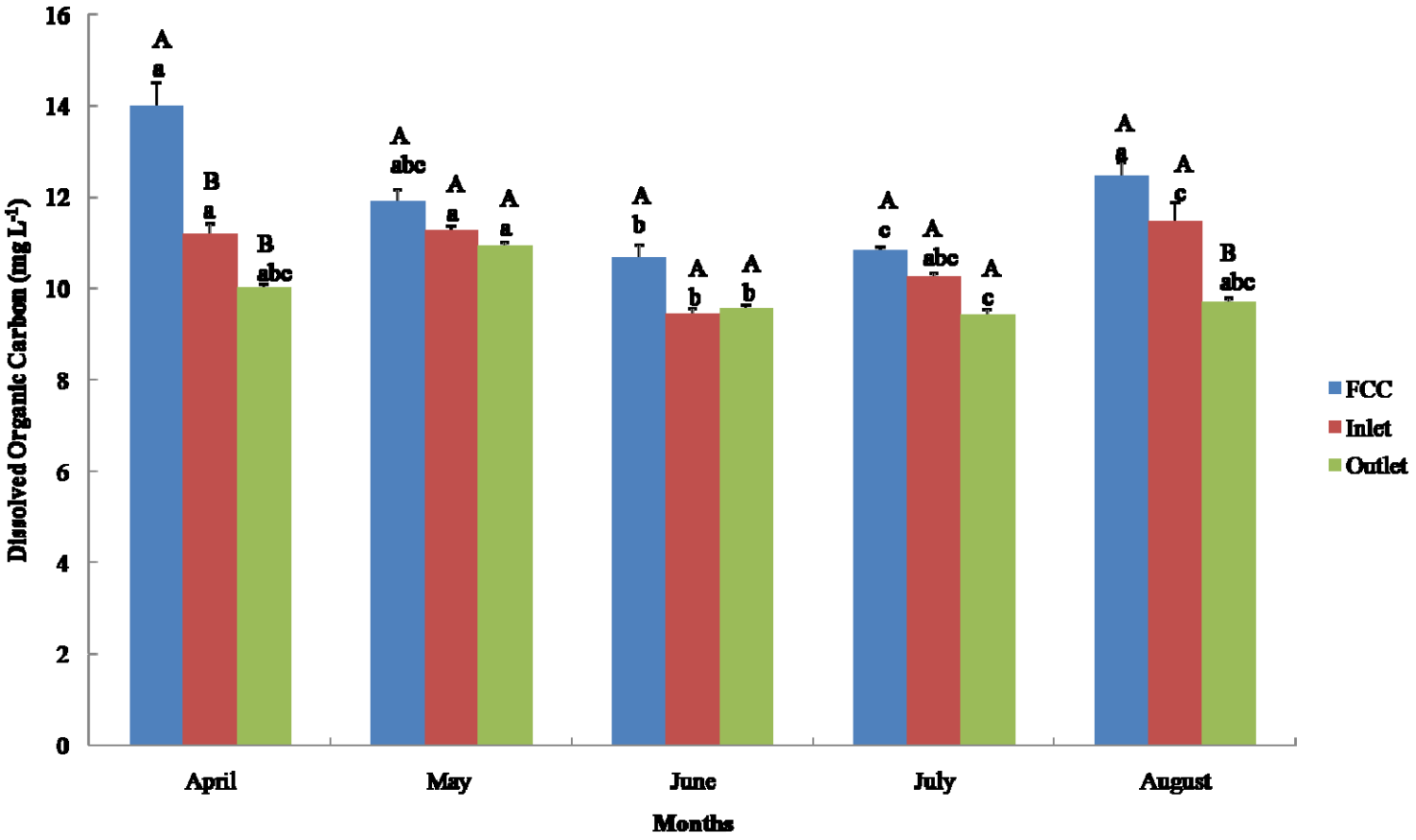

Figure 3-6. Mean concentration (mg L^{-1}) of dissolved organic carbon (DOC) between April 2007 and August 2007 in the flow control channel (n=3), inlet (n=9) and the outlet (n=9) of the study cell of a constructed wetland receiving nursery runoff. Error bars indicate plus standard error. Capital letter indicates statistical difference between locations within each month (May to August); lower-case letters indicate statistical difference between months at each location (FCC, inlet and outlet). Means followed by the same letter are statistically similar.

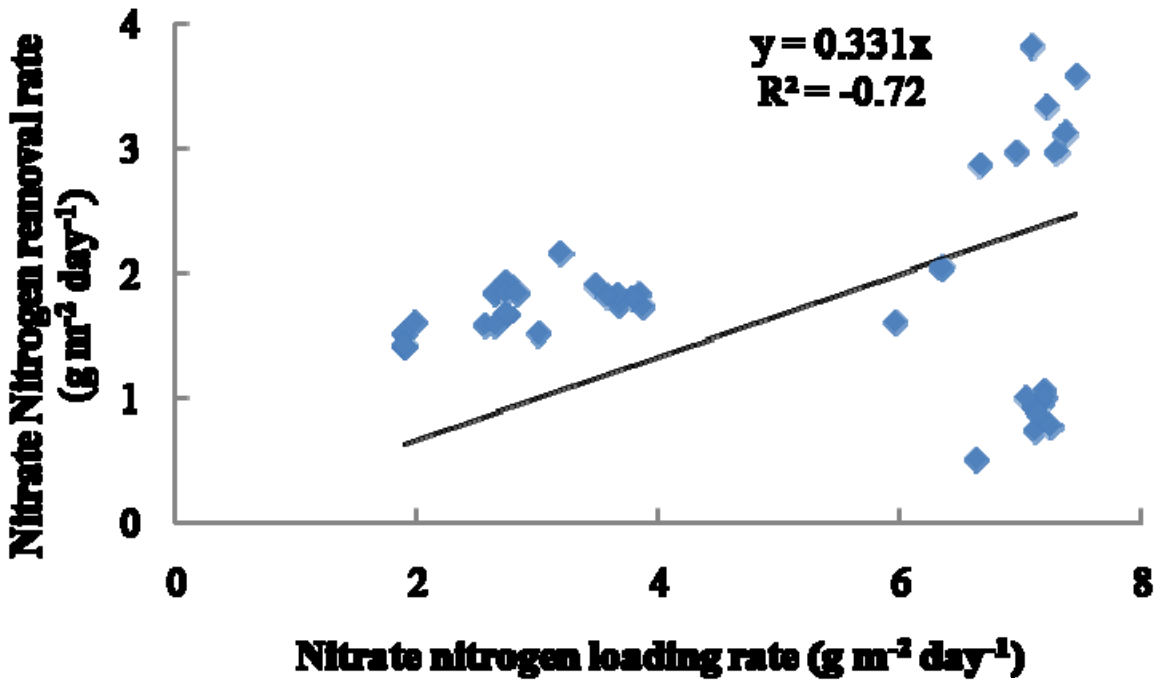

Figure 3-7. Pearson's correlation between loading and removal rate of nitrate nitrogen (g m^{-2} day^{-1}) between April 2007 and August 2007 in a constructed wetland receiving nursery runoff.

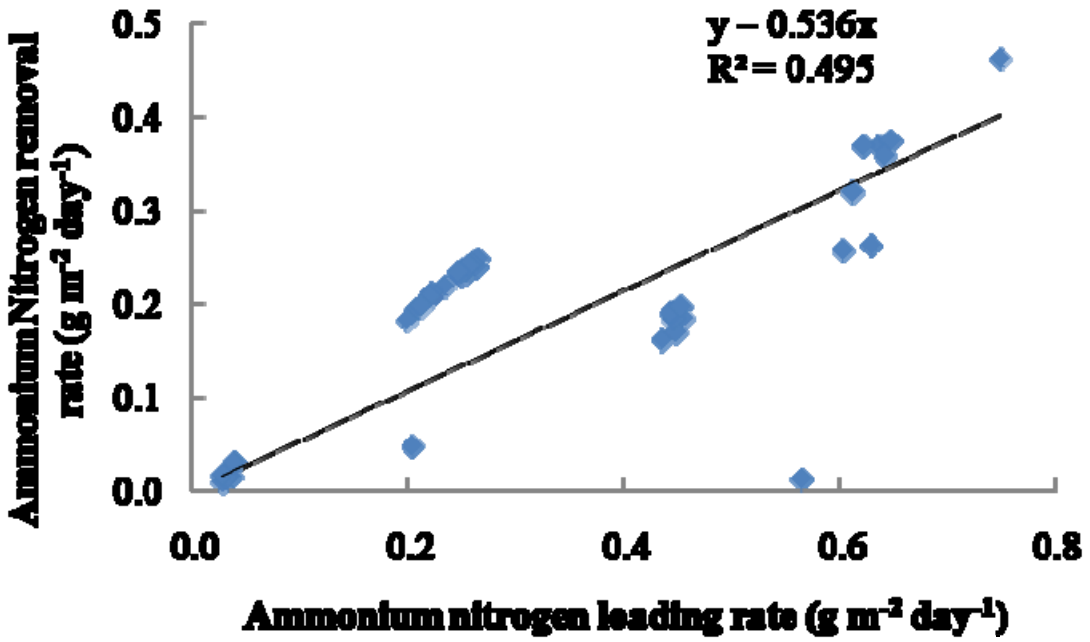

Figure 3-8. Pearson's correlation between loading and removal rate of ammonium nitrogen (g m^{-2} day^{-1}) between April 2007 and August 2007 in a constructed wetland receiving nursery runoff.

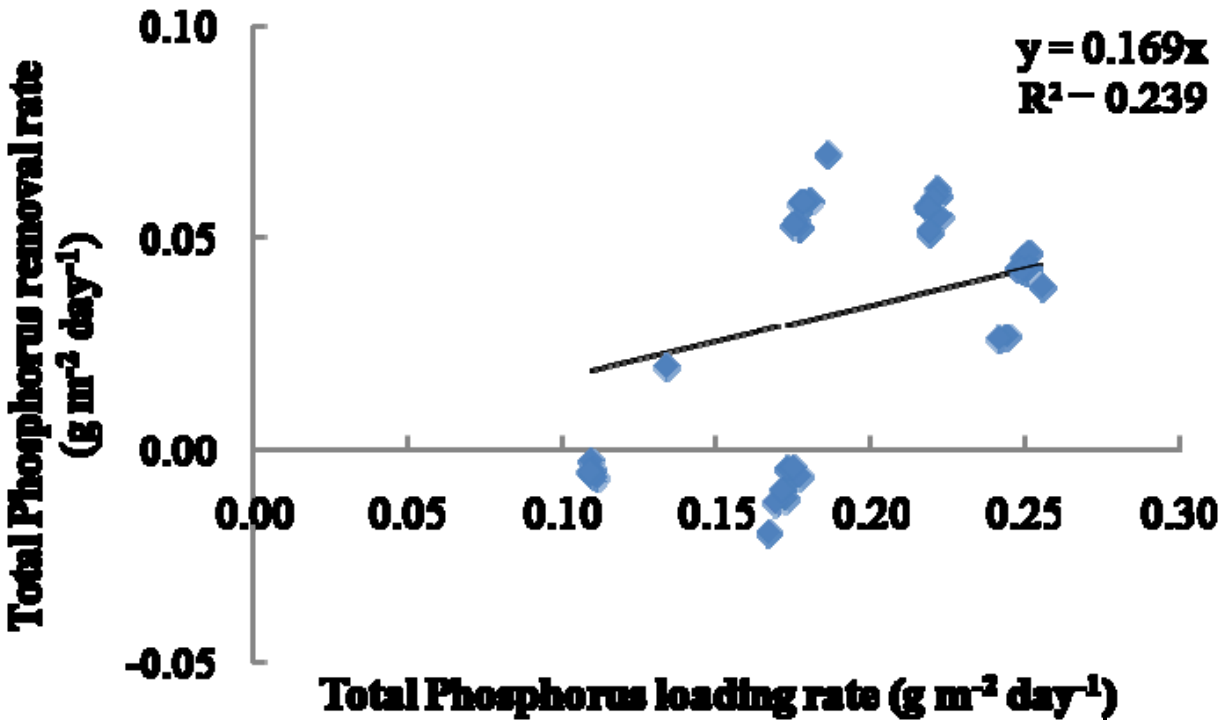

Figure 3-9. Pearson's correlation between loading and removal rate of total phosphorus (g $m^{-2}day^{-1}$) between April 2007 and August 2007 in a constructed wetland receiving nursery runoff.

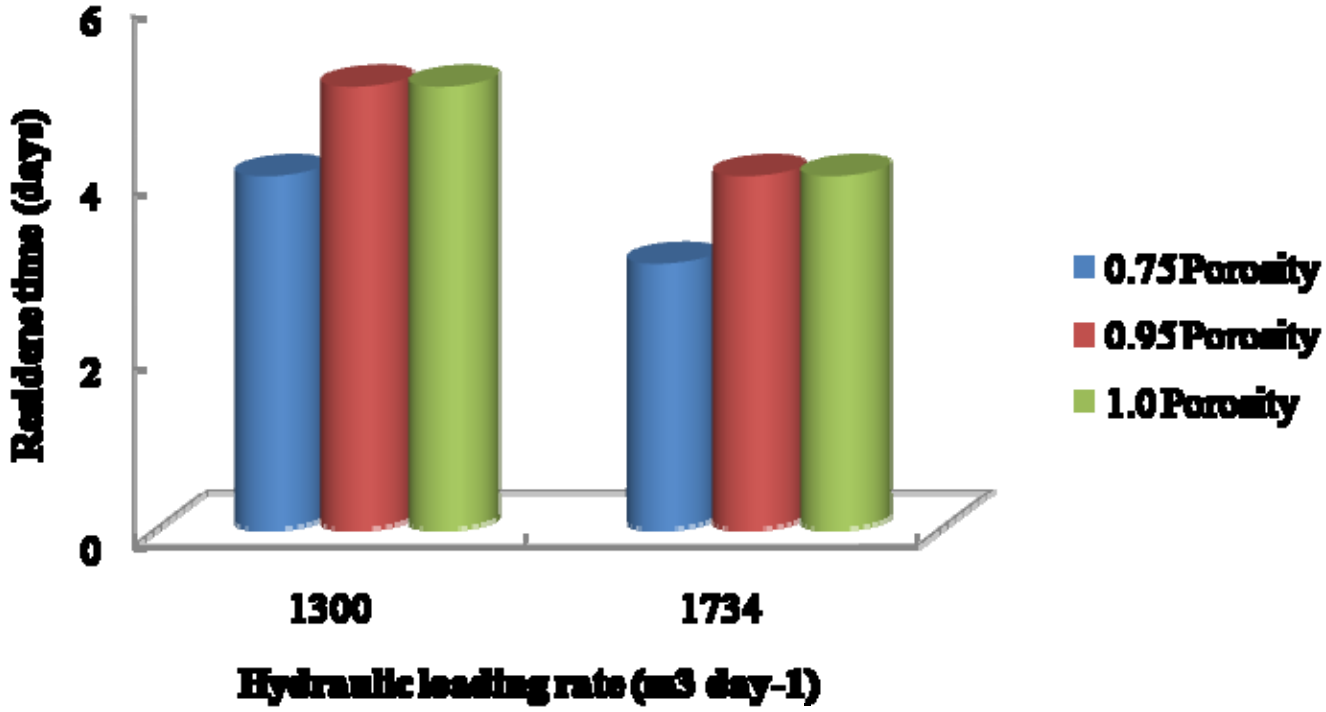

Figure 3-10. Theoretical hydraulic residence time (in days) as affected by hydraulic loading rate (m^3 day^{-1}) and three estimates of wetland porosity values (0.75, 0.95 and 1.0) in the study cell of a constructed wetland receiving plant nursery runoff

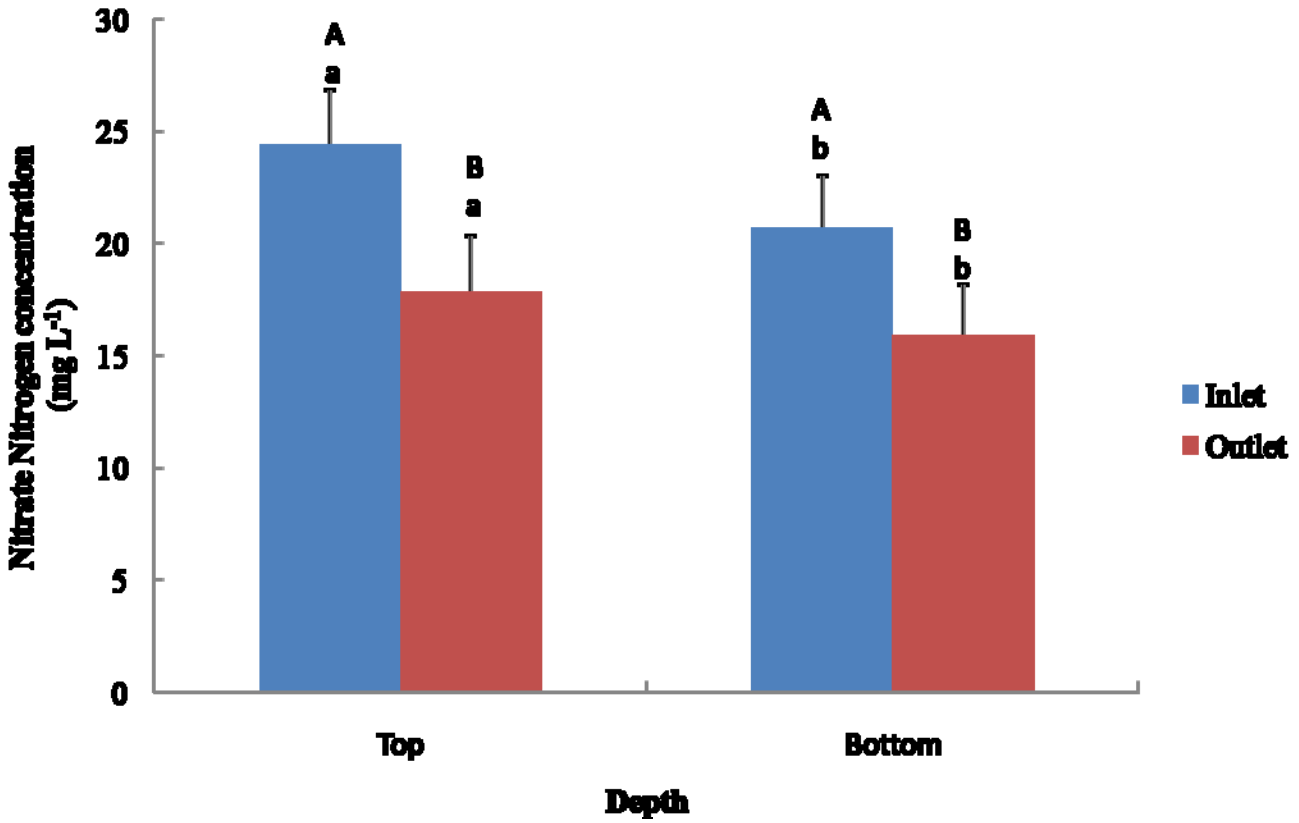

Figure 3-11. Mean concentrations of nitrate nitrogen (mg L^{-1}) as affected by location of sample collection and depth within location, between April and August 2007 in the constructed wetland receiving nursery runoff (n=18). Capital letters indicate statistical difference between location (inlet and outlet) at top and bottom of the water column; lower-case letters indicate statistical difference between depths (top and bottom) at inlet and outlet over five months. Means followed by the same letter are statistically similar.

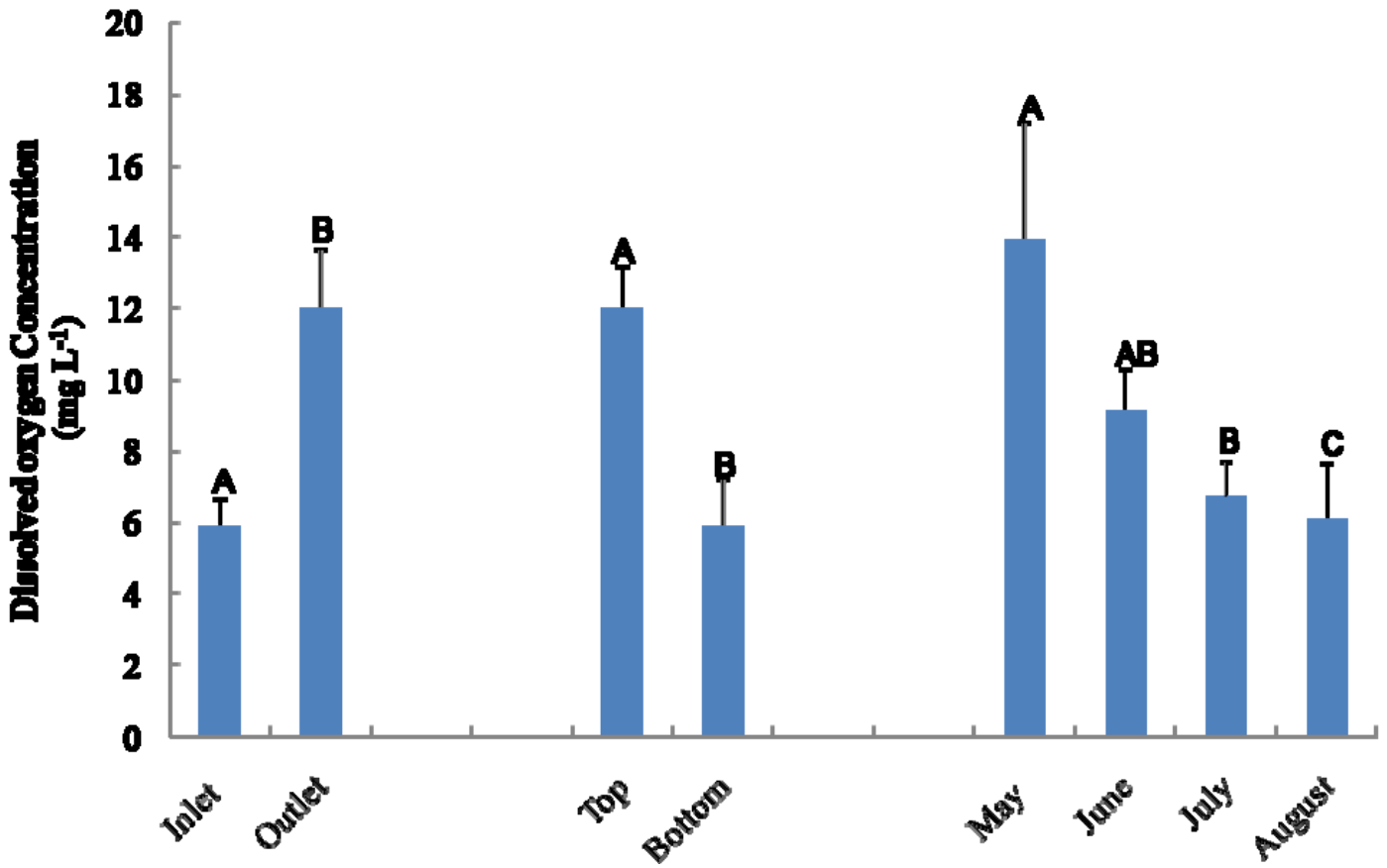

Figure 3-12. Dissolved oxygen concentration (mg L^{-1}) as affected by location of sample collection, depth within location, and months (April and August 2007) in the constructed wetland receiving nursery runoff (n=3). Capital letters indicate statistical difference within location, within depth, and within months. Means followed by the same letter are statistically similar.

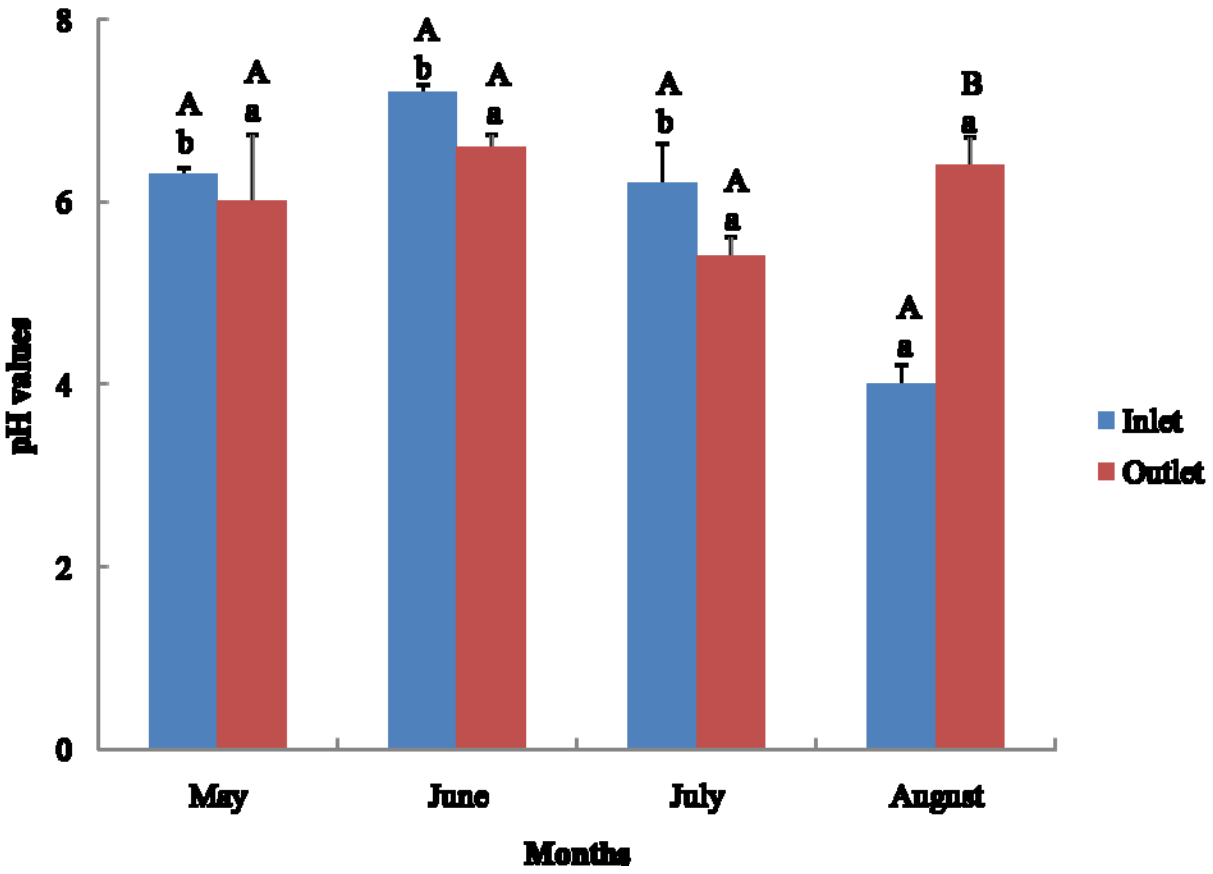

Figure 3-13. Physiochemical parameter, pH, as affected by location of sample collection, and months (April and August 2007) of the constructed wetland receiving nursery runoff (n=3). Capital letter indicates statistical difference between locations within each month (May to August); lower-case letters indicate statistical difference between months at each location (inlet and outlet). Means followed by the same letter are statistically similar.

CHAPTER 4
DISCUSSION

Concentration of nutrients in the inflow and outflow water. The mean concentration (over all locations and months) of nutrients in plant nursery runoff entering the constructed wetland were 34 mg L^{-1} total nitrogen (TN) [30 mg L^{-1} nitrate nitrogen (NO_3^- - N); 2 mg L^{-1} ammonium nitrogen (NH_4^+-N); and 2 mg L^{-1} organic nitrogen], 1 mg L^{-1} total phosphorus (TP), and 10 mg L^{-1} Dissolved Organic Carbon (DOC). The concentration of DOC, less than 20 mg L^{-1}, is considered low as compared to other wastewater sources (Headley et al., 2001; Huett et al., 2005; Crumpton et al., 1993). The nutrient removal efficiency of the constructed wetland was observed to be 40% for TN, 40% for NO_3^- - N, 59% for NH_4^+-N, and 16% for TP. The constructed wetland systems in the US are reported to provide removal efficiencies of 66-95% for TN and TP (Dortch, 1992), 30-60% for N (Hammer and Knight, 1994). Headly et al. (2001) have reported > 84% TN and > 65% TP removal in the subsurface horizontal flow wetlands receiving plant nursery runoff. Huett et al. (2005) obtained > 95% removal efficiency for both TN and TP in a subsurface flow wetlands treating plant nursery runoff. Reinhardt et al. (2005) reported 23% TP removal efficiency in constructed wetlands treating agricultural drainage water. While comparing the results, the study cell appears to be functioning moderately efficiently in removing TN and TP.

Mean removal efficiency for NO_3^- - N of the constructed wetland over five months was 40%. Nitrate nitrogen removal efficiency of treatment wetlands in the US ranges from 30-60% (Hammer and Knight, 1994). Poe et al. (2003) obtained 53% of NO_3^- - N removal in a constructed wetlands treating agricultural runoff. It is possible that a longer sampling period at the study cell may have given different results.

The removal efficiency of the NO_3^--N during the study period ranged from 12 -77% over five months, indicated the temporal variation in effectively removing $NO3^-$ - N. The highest NO_3^- - N removal was obtained in the month of August 2007 and the lowest removal efficiency was obtained during April. During the same period, the concentration of NO_3^- - N entering and leaving the wetland gradually decreased from April to August. There was an inverse relationship between the concentration of NO_3^- - N at inflow and the removal efficiency of the wetland, i.e., when the concentration of NO_3^- - N entering the wetland was higher, the removal efficiency was lower during April. Similar relationship was observed between loading rates and removal efficiency of NO_3^- - N in the constructed wetlands. Gradual increase in removal efficiency of NO_3^- - N from April (12%) to August (77%) was observed, as the inflow concentration gradually decreased from 47 mg L^{-1} to 13 mg L^{-1}. Lin et al. (2002) observed similar decrease in the efficiency of nitrate removal but the rate of removal increased as the nitrate loading rate was increased. Darbi et al. (2003) also reported inverse relationship between loading rate and the removal efficiency of NO_3^--N. Reduction in the removal efficiency of NO_3^- - N from 2.46 to 1.64 kg NO_3^--N m^{-3} day^{-1} was obtained, as the inflow NO_3^--N concentration increased from 175 to700 mg L^{-1} (Park et al., 2002). Most of the studies reported high loads of NO_3^--N as compared to the load in the present study. As the loading rate of NO_3^--N was observed to be less than 7 g m^{-2} day^{-1}, this study provides a useful insight into nutrient removal at lower loading rate. Similar results of low NO_3^--N loading rates were observed (<2 g m^{-2} day^{-1}) by Headly et al. (2001) and Huett et al. (2005) in subsurface flow reed beds receiving plant nursery runoff in Australia. Although the loading rates are comparatively low, the removal rate of NO_3^- - N (1.75 g m^{-2} day^{-1}) in this study is comparable and in many cases higher as compared to the values of other studies (Table 4.1).

The Pearson correlation coefficient value (r = 0.19) indicated a little/no association between loading and removal rate of NO_3^- - N. The strong positive association between loading and removal rate of NH_4^+-N (r = 0.75) and a weak positive association between loading and removal rate of TP (r = 0.6). The positive correlation coefficient indicates that the loading and removal rate tend to increase or decrease together. Similar linear relationship of increasing removal rate with increasing loading rate has been observed by Headley et al. (2001) for TN (r = 0.76).

Plant nursery runoff in this study consisted 87% of the TN in the form of NO_3^- - N. The dominance of NO_3^- - N in runoff water entering the wetland could be attributed to the controlled release (coated urea), and soluble nitrate fertilizers (Ammonium Nitrate) applied to nursery plants (S. Chandler, Horticulture Manager Monrovia Nursery personal communication). It is not uncommon to apply up to 200 mg L^{-1} nitrate nitrogen to ornamental crops during peak growing season. The concentration of NO_3^- - N (mg L^{-1}) entering the wetland varied during the period of study, i.e., it gradually decreased from April to August 2007. The higher concentration of NO_3^- - N during April was likely due to fertilization practices at the nursery during their peak growing season (S. Chandler, Horticulture Manager Monrovia Nursery personal communication). In addition, the limited rainfall (15-17 cm during April and May 2007) might have contributed to increased concentration of nutrients in the runoff. Similarly, increased concentration of nutrients in the nursery runoff was observed due to fertilization in subsurface horizontal flow reed beds in subtropical region of Australia (Headley et al., 2001). During April and May there were comparatively higher concentrations (44 mg L^{-1} and 36 mg L^{-1}, respectively) of NO_3^- - N entering the wetland as compared to June, July and August. Higher concentration of NO_3^- - N during April (averaging 19 mg L^{-1}) and May (averaging 18 mg L^{-1}) were also obtained by Taylor

et al. (2006) during their four year (2002 – 2005) monitoring of influent of the constructed wetland at Monrovia Nursery. In addition, application of fertilizers at a nearby horticultural nursery contributed to the increase in the concentration of NO_3^- - N (0.03 to 4.73 mg L^{-1}) through runoff in the tributaries of Santa Fe river watershed in north central Florida (Frisbee, 2007).

While assessing the composition of TN during the study period, it was observed that concentration of organic N at the outflow was higher as compared to inflow of the study cell, which might be due to internal generation of organic N in the wetland during decay of plant and microbes. Persistence of organic N in low concentrations was always observed in the outflow water of a constructed wetland (Kadlec and Knight, 1996; Headley et al., 2001).

Water temperature in study wetland was observed to gradually increase from May to August. The NO3--N removal efficiency was observed to gradually increase during the same period. Nitrate nitrogen removal might have been enhanced by the increase in temperature, which probably resulted in vigorous plant growth and activity of the microorganisms. The mean temperature of the wetland (over all locations, depths and months) was 26^0C. The favorable range of temperature for denitrification is between 20 and 25^0C (Spieles and Mitsch, 2000), which is close to the range of the mean temperature of the study cell. The influence of temperature on nitrogen removal was explained by Reddy and Patrick (1984). The rates of denitrification have been shown to increase by 1.5-2 times with each increase in 10oC. Water temperatures less than 15°C or greater than 30°C have been shown to limit the rate of denitrification (Reddy and Patrick, 1984).

The gradual increase in NO_3^--N removal efficiency over April to August could also be related to the growth of the plants in the wetland. The perennial plants in the wetland started growth in April. During the vigorous growing stages (April, May, and June), plants utilize NO_3^--

N and thereby may have contributed to the increased removal efficiency of the NO_3^- - N. Studies have shown that macrophytes in the wetlands remove substantial amount of NO_3^--N directly by uptake or indirectly by providing carbon exudates from roots, which enhance microbial transformations (Gersberg et al., 1983; Bachand and Horne, 2000).

The NH_4^+-N contributed only less than 1% of the TN load into the wetland. The removal efficiency of the NH_4^+-N ranged from 41% (June) to 93% (August). The concentration of NH_4^+-N coming into the wetland was approximately 2 mg L^{-1}, and the outflow concentration less than 1 mg L^{-1}. The NH_4^+-N removal is likely because of plant uptake or nitrification process in the aerobic sites of the wetlands. Immobilization by plants and microbes enhances the removal of NH_4^+-N. In addition, microbe mediated nitrification process, in which NH_4^+-N is oxidized to NO_3^- - N also contributes to NH_4^+-N removal in the aerobic zones of wetlands (Burger and Jackson, 2004). The volatilization of NH_4^+-N to ammonia would not be a significant process for NH_4^+-N removal in this wetland as the mean pH value (6.0) was lower than optimal (> 8) for this chemical process to occur (USEPA, 1993, Sartoris et al., 2000). The pH values in this system did not reach that optimum level during the study period. Plant growth and the size of denitrifier population within the rhizosphere are observed to be coupled. Hence the influence of pH on plant growth in turn affects the denitrification potential (Hall et al., 1998). However, the influence of pH on plant growth was not studied in this system during this study.

The concentration of TP entering and leaving the wetland cell was approximately 1 mg L^{-1}. The removal efficiency of TP ranged from 0-32%, which varied over months. The removal of TP (which consists orthophosphate) may be attributed to biological uptake, adsorption onto sediment, etc. The percent TP removal efficiency over months did not follow any particular trend, however. Similar results of variable TP net removal during the growing season were

observed by Taylor et al. (2006) in the constructed wetland at Monrovia nursery. The few negative removal efficiency values of TP indicate that there is likely internal loading of phosphorus. Macrophytes, algae, and microorganisms utilize P as an essential nutrient and can enhance temporary removal of TP. Long term TP removal, however, is limited to the P sorption capacity of the sediments (Kadlec, 1999).

The concentration of DOC did not follow a particular trend over months. The distribution of DOC seemed uniform through the wetland in the study cell. The nursery runoff had a low dissolved organic carbon (DOC) concentration (a characteristic of nursery runoff). The low dissolved organic carbon (DOC < 20 mg L^{-1}) was observed in the subsurface flow reed bed systems receiving plant nursery and agricultural runoff (Headley et al., 2001; Huett et al., 2005; Crumpton et al., 1993) in Australia. The DOC: NO_3^- - N ratio was observed to be low (<3:1) during most of the study period.

The modeled effect of hydraulic loading rate and plant density on theoretical residence time was noticeable. When higher values of hydraulic loading rate were used in the model, the theoretical residence time decreased by a day for this system. The model showed that increasing the wetland porosity from 0.75 to 0.95 and/or 1.0 would increase the residence time in the study cell by one day. Based on the model, the theoretical residence time for this system ranged from 3 to 5 days. Headley et al. (2001) reported that the maximum N removal can be achieved even at 2 day residence time in the subsurface flow reed beds. It was estimated that a nursery of 1 ha area would require a reed bed area of 200 m^2 for a 2 day hydraulic residence time.

Spatial effects on nutrients, physiochemical parameters and denitrification potential of the water column. The higher DO level at outflow can be attributed to the higher prevalence of open water towards the outflow side of the wetland compared to the inflow side. The depth of

the water column showed significant influence on DO level, which is expected in waterlogged conditions. The anoxic condition of the wetland was enhanced with increasing depth of the water column. The DO concentration gradually decreased from April to August, when the temperature gradually increased from April to August. The reduction in concentration of DO could be attributed to increased activity of microorganisms, which enhances N removal, with the gradual increase in temperature.

The nutrients (N and P) analyzed in this study varied in their concentration at inflow and outflow. This variation in the concentration of nutrients may be attributed to removal of nutrients within the study wetland. Similar nutrient gradient from the inlet to outlet points was observed in Everglades's wetlands soil (White and Reddy, 1999) and in experimental wetlands receiving agricultural runoff (Sirivedhin and Gray, 2006). The low concentration of NO_3^- - N at outflow is likely due to the removal of nitrogen by various mechanisms such as plant uptake, microbial assimilation, and denitrification along the flow of water from inflow. The low concentration of NO_3^- - N at lower depth of the water column is likely due to the removal of NO_3^- - N by plant uptake and denitrification process in the anaerobic zones of the water column. Another possible reason might be the lack of diffusion of NO_3^- - N to the lower depths of the water column, which will also limit the denitrification activity.

Although the concentration of NH_4^+-N was always higher at inflow as compared to outflow of the wetland, there was no trend observed in the spatial distribution of NH_4^+-N within the wetland cell over the period of five months. The lower concentration of NH_4^+-N at outflow could be attributed to the removal of NH_4^+-N within the study cell. However, the concentration of NH_4^+-N was observed to be higher in lower depth of the water column, which is likely due to lack of nitrification in the anoxic zones of the water column.

Significant spatial distribution in total phosphorus concentration was observed within the wetland. However, there were no temporal effects on TP concentration during the period of this study. The TP was observed to be higher in the deeper water column as compared to the surface of the water column, but only at the inflow side of the wetland. This could be due to the release of adsorbed P from the sediments into immediate atmosphere, or due to lack of adsorption sites near the inflow of the study cell. The sorption sites on the inflow side might have become saturated because of the higher concentration of TP at inflow. The DOC concentration did not show any differences in its distribution within the depth of the water column, suggesting its uniform distribution within the wetland.

The denitrification potential of the water column in the study cell was either negligible or undetectable. The maximum value of denitrification potential observed in the water column was 0.03 mg N_2O-N L^{-1} hr^{-1}. Lower values of denitrification potential are likely due to a lack of particulate material in the water column to support the microbial activity. Little research has been conducted on denitrification potential of the water column in constructed wetlands, but whenever it was included in wetland studies, the reported values were always relatively low. Denitrification rates of the water samples in a constructed wetland receiving sewage treatment plant effluent were observed to be between 0.06 – 0.96 µg N m^{-2} d^{-1} (Toet et al., 2003). The water column of the study cell had low dissolved organic carbon (DOC) concentration, and this may be limiting NO_3^- - N removal due to non availability of energy for denitrifying microbes. The optimum C:N (plant carbon added:NO_3^--N in water) ratio for the denitrification in wetlands is reported to be 4:1 (Ingersoll and Baker, 1998) to 5:1(Baker, 1998). Only during the month of April, the DOC: NO_3^- - N ratio reached near 4:1 in the wetland. The mean DOC: NO_3^- - N ratio was low (<1) during most of the study period. Low DOC: TN reported to limit NO_3^- - N removal

in a subsurface flow wetland receiving plant nursery runoff (Huett et al., 2005). The absence of attachment sites for bacteria and/or low organic carbon availability possibly resulted in low denitrification rates in the water, as reported by Toet et al. (2003). The distribution of denitrifying microbes was most likely regulated by the availability of organic material, with higher denitrification rates in the sediments than on surface in the water column. The DO concentration in the water column of the study cell ranged from 6 mg L^{-1} to 12 mg L^{-1}, which might have affected the denitrification process, as well. Poe et al. (2003) reported that the denitrification potential was influenced by the concentration of NO_3^--N, and the rates of denitrification were observed to increase with an increase of NO_3^- - N. Such trend was not observed in this study.

Denitrification potential of macrophyte rhizosphere soil. Denitrification potential of macrophyte rhizosphere soil was influenced by months; however, there was no trend between April and August 2007. The mean denitrification potential in the rhizosphere of the two species combined was 7.6, 1.7, 0.9, and 3.0 mg N_2O-N kg^{-1} hr^{-1}, in May, June, July, and August 2007, respectively. Only the values obtained in May significantly differed from other months. Although there was a gradual increase in temperature of the water column from May (22˚C) to August (30˚C), the rhizosphere denitrification potential did not show significant difference over months. The mean temperature (26˚C) of the wetland during the study period was close to the optimum range, 20-25˚C, for denitrification activity.

Results of this study showed that mean denitrification potential over five months for *Canna flaccida* and *Typha latifolia* was 3 mg and 4 mg N_2O-N kg^{-1} hr^{-1}, respectively. Data variability within replicates was observed for denitrification potential values in this study. The variation in values obtained for replicates in this study might be due to the heterogeneity in the

sampling point as affected by plants and surrounding environment, water movement, etc. Similar data variability in the denitrification rates between the replicates was observed by Bastviken at al. (2003).

Regardless, the denitrification values obtained in this study are relatively high when compared to several other studies (Table 4-2). Hunt et al. (2003) obtained higher denitrification potential of 0.21 mg N kg^{-1} hr^{-1} in *Typha* dominated wetland soils, as compared to 0.52 mg N kg^{-1} hr^{-1} in *Juncus* dominated wetlands used for treatment of swine wastewater. Denitrification potential values observed in this study also lie in the range of denitrification potential (0.004 – 7.75 mg N kg^{-1} hr^{-1}) obtained for everglades wetland soils (White and Reddy, 1999). Denitrification rates in soil samples from *Juncus*-planted wetland plots were reported to be higher (12.3 mg N m^{-2} d^{-1}) as compared to *Salix* plots (2.65 mg N m^{-2} d^{-1}). The denitrification rates obtained from soil samples amended with nitrate and/or glucose, indicated that the denitrification was limited by the availability of carbon and NO_3^- - N in the *Salix* plots, whereas only by NO_3^- -N in the *Juncus* plots (Smialek et al 2006). Denitrification potential in rhizosphere soil of *Lolium perenne* L.Melinda (Rye grass) was observed to be 150 – 900 ng N g^{-1} hr^{-1}, and followed the pattern of plant growth, indicating that the plant growth and the size of denitrifier population within the rhizosphere are likely correlated (Hall et al., 1998). Ottosen et al. (1999) observed low denitrification activity in the rhizosphere of *Zostera marina* and *Potamogeton pectinatus* (1.5 to 5 μmol N m^{-2} h^{-1}) as compared to *Lobelia dortmanna* and *Littorella uniflora* vegetated sediments (24 and 30 μmol N m^{-2} h^{-1}).

Statistically similar denitrification potential in *Canna flaccida* and *Typha latifolia* in this present study might be due to their similar effect on the rhizosphere. Other researchers have observed differences in denitrification potential in wetland soil and sediments planted with

different taxa of plants. These differences were attributed to differences in the ability of plant roots to diffuse oxygen into the rhizosphere, and also due to differences in leakage of available organic carbon from plant roots.

Most of the studies on denitrification potential have used the sediments and bulk soil in the rhizosphere of the plants as substrate. Results from this study show that denitrification rates comparable to or higher than those reported from bulk soil of vegetated plots can be obtained in rhizosphere soil closely adhered to the roots.

Table 4-1. Nitrate removal rates in constructed wetlands, under different hydraulic residence periods, as reported by other researchers.

System	Wastewater	Hydraulic residence time (days)	Nitrate removal rates ($g\ m^{-2}\ day^{-1}$)	
Flow through wetland microcosms	Nitrate contaminated water	2.4	2.8-5	Ingersoll and Baker, 1998
Constructed free water surface wetlands	Landfill leachates	10-12	0.63	Kozu and Lieh, 1999
Constructed wetlands	Agricultural tile drainage	7	0.29–1.51	Xue et al., 1999
Constructed free water surface wetlands	River flow	1-10	0.56	Bachand and Horne, 2000
Natural forested treatment wetland	Municipal wastewater	0.9 – 1.1	0.10	Blahnik and Day, 2000
Constructed wetlands	Groundwater	4.2	0.94	Lin et al., 2002
Subsurface flow constructed wetlands	Domestic effluent	10.5	0.61	Bayley et al., 2003
Surface flow constructed wetlands	Nursery runoff	3-5	1.75	This study

Table 4-2. Denitrification potentials of different substrate reported by other researchers in constructed wetlands. While the units of measure are different and make direct comparisons challenging, these data are useful for making general inferences for wetlands dominated by different plant taxa. The acronyms used are as follows: Denitrification Enzyme activity (DEA), Acetylene Inhibition Technique (AIT), Membrane Inlet Mass Spectrometry (MIMS), and N^{15} tracers (N^{15}).

Substrate	Wastewater type	DEA ($mg\ N\ m^{-2}\ d^{-1}$)	Range of DEA values	Method	Reference
Phragmites australis shoots	Sewage treatment plant effluent		44.4 - 121 $mg\ N\ m^{-2}\ d^{-1}$	AIT	Toet et al., 2003
Elodea nuttallii shoots	Sewage treatment plant effluent		14.8 – 33.1 $mg\ N\ m^{-2}\ d^{-1}$	AIT	Toet et al., 2003
Sediments	Sewage treatment plant effluent		0.5 – 25.5 $mg\ N\ m^{-2}\ d^{-1}$	AIT	Toet et al., 2003
Water	Sewage treatment plant effluent		0.4 – 3.9 $\mu g\ N\ m^{-2}\ d^{-1}$	AIT	Toet et al., 2003
Spartina spp., *Cladium* spp. *Juncus* spp. sediment cores	Agricultural runoff	2.5	0.7 – 9.2 $mg\ N\ m^{-2}\ d^{-1}$	MIMS	Poe at al., 2003
Juncus spp. sediment	Agricultural runoff	2.3	0.1-6.0 $mg\ N\ m^{-2}\ d^{-1}$	AIT	Thompson et al., 2000
Everglades soils	Agricultural runoff		0.004 – 7.75 $mg\ N\ kg^{-1}\ hr^{-1}$	AIT	White and Reddy, 1999
Mixed plants sediments	Agricultural tile drainage		2-11.8 $mg\ N\ m^{-2}\ d^{-1}$	AIT /N^{15}	Xue et al.,1999
Lolium perene (Ryegrass) Rhizosphere soil	Wetland microcosms		150 – 900 $ng\ N\ g^{-1}\ hr^{-1}$	AIT	Hall at al., 1998
Typha latifolia	Plant nursery runoff	2.51 $mg\ N\ kg^{-1}\ hr^{-1}$		AIT	This study
Canna flaccida	Plant nursery runoff	4.11 $mg\ N\ kg^{-1}\ hr^{-1}$		AIT	This study

CHAPTER 5
SUMMARY AND CONCLUSIONS

Constructed wetlands are cost effective and viable method for on-site removal of nonpoint source contaminants from water before it is released into aquatic systems. These systems are used to treat wastewater from municipal water source, animal waste, urban runoff, stormwater runoff, and agricultural runoff. Few studies have focused on constructed wetlands treating nursery runoff (Headley et al., 2001; Lea-cox et al., 2002; Huett et al., 2005). In general, the performance of constructed wetlands varies and partly depends on the source and the characteristic features of influent wastewater (Mitsch and Jorgensen, 1989; NRCS, 2000a; Dunne et al., 2005).

When constructed wetlands are to be used for nutrient, especially N, removal, management strategies can be optimized by understanding the mechanisms by which N is removed within the constructed wetlands. In view of the necessity of a 'systems approach', which recognizes the site specific conditions, nitrogen (N) dynamics in the constructed wetland receiving plant nursery runoff was investigated to facilitate improved understanding of N transformations, factors influencing N removal, and to optimize the performance of the system for on-site N removal.

The concentration of nutrients N and phosphorus (P) entering and leaving the constructed wetland was monitored from April 2007 to August 2007 to determine the nutrient removal efficiency of the study cell in the constructed wetlands receiving plant nursery runoff. The dominant nutrient in the plant nursery runoff was N, in the form of nitrate nitrogen (NO_3^--N), followed by phosphorus (P). The concentration of DOC was low (< 20 mg L^{-1}) which is a characteristic feature of the plant nursery runoff.

It was hypothesized that the concentration of N and P would vary over months based on the timing of the fertilizer application, irrigation, and rainfall. The higher concentration of NO_3^--

N during April and May in this study was likely due to fertilization practices at the nursery during their peak growing season. In addition, the limited rainfall (15-17 cm during April and May 2007) might have contributed to increased concentration of nutrients in the runoff.

The concentration of nutrients, as expected, was higher in the influent as compared to the outflow. The mean nutrient removal efficiency of the constructed wetland was 40% for TN, 40% for NO_3^--N, 59% for NH_4^+-N, and 16% for TP. While comparing to other studies, the study cell appears to be functioning moderately efficiently in removing N and P. The NO_3^--N removal efficiency was inversely related to the loading rate of NO_3^--N in this study. The P removal might be due to plant, algal uptake and adsorption/precipitation reactions. The negative P removal efficiency in July indicates that at times there may be internal loading of P in the system. Ammonium Nitrogen removal is likely due to plant uptake or nitrification process in the aerobic zones of the wetlands. The volatilization of NH_4^+-N to ammonia is probably not a significant process for NH_4^+-N removal in the wetland because the observed pH in the wetland was lower that what is reported to be optimal for this process to occur. The low DOC: NO_3^--N ratio in the influent water indicates that the system might be limited by carbon availability, which could adversely affect NO_3^- - N removal.

Hydraulic loading rate and the plant density were observed to influence the theoretical residence time in the model. When higher values of hydraulic loading rate were used in the model, the theoretical residence time decreased by a day for this system. The model showed that increasing the wetland porosity from 0.75 to 0.95 and/or 1.0 would increase the residence time in the study cell by one day.

The nutrients (N and P) analyzed in this study exhibited a gradient from the inflow to outflow due to removal of nutrients within the wetland. The low NO_3^--N concentration at lower

depth of the water column is likely due to utilization of NO_3^--N by microorganisms, during the process of denitrification in the anoxic zones. The high NH_4^+-N concentration in the lower depth of the water column is likely due to the lack of nitrification process in the anoxic lower depth of the water column, and hence the accumulation of NH_4^+-N. Higher concentration of TP in the lower depth of the water column is likely due to release of adsorbed P from the sediments, or due to lack of adsorption sites near the inflow of the study cell. The sorption sites on the inflow side might have become saturated because of the higher concentration of TP at inflow. Concentration of DOC did not show any differences in distribution along the depth of the water column, and across the locations over months, indicated uniform distribution within the wetland.

Gradual increase in the temperature of the water column from May to August 2007 might have enhanced NO_3^- - N removal by affecting plant growth and activity of the microorganisms. Denitrification potential values were observed to follow the pattern of plant growth, indicating that the plant growth and the size of denitrifier population within the rhizosphere might be coupled. Hence, physiological parameters affecting the plant growth can also indirectly affect denitrification. A direct relationship was observed between the NO_3^- - N removal efficiency and temperature of the water column during the study period. As the temperature gradually increased from May to August, the DO level gradually decreased. This is likely due to the enhanced activity of the microorganisms utilizing oxygen as an electron acceptor. The higher DO level at outflow can be attributed to open water towards the deeper side of the wetland. Due to the potential differences in the temperature over months, DO level in the water column, and concentration of NO_3^--N at inflow and outflow, it was expected that the denitrification potential of the water column would be different as well. However, the low (0.01-0.03 mg N_2O-N L^{-1} hr^{-1}) or undetectable denitrification potential of the water column is possibly due to the low DOC

concentration/low DOC: NO_3^--N, which limits denitrification. The presence of few attachment sites for bacteria possibly resulted in low denitrification rates in the water column.

Mean rhizosphere denitrification potential over five months for *Canna flaccida* and *Typha latifolia* was 3 mg and 4 mg N_2O-N kg^{-1} hr^{-1}, respectively. The mean denitrification potential of two species was statistically similar. Regardless, the denitrification values obtained in this study are relatively high when compared to several other study studies. Hunt et al. (2003) obtained higher denitrification potential of 0.21 mg N kg^{-1} hr^{-1} in *Typha* dominated wetland soils, as compared to 0.52 mg N kg^{-1} hr^{-1} in *Juncus* dominated wetlands used for treatment of swine wastewater. Rhizosphere denitrification potential values of Canna *flaccida* and *Typha latifolia* in this study were comparatively higher than the rhizosphere denitrification potential values of other plant species *Lolium perenne*, *Zostera marina*, *Potamogeton pectinatus*, *Lobelia dortmanna* and *Littorella uniflora*. Results from this study show that denitrification rates comparable to or higher than those reported from bulk soil of vegetated plots can be obtained in rhizosphere soil closely adhered to the roots.

Nitrate nitrogen removal efficiency of the constructed wetland receiving plant nursery runoff was observed be 40% over five month period, with an average removal rate of 1.75 g m^{-2} day^{-1}. The mean loading rate of NO_3^--N was 4.43 g m^{-2} day^{-1}. Compared to mean removal rate of NO_3^--N (1.75 g m^{-2} day^{-1}), the amount of NO_3^--N removed through coupled (0.1 g N_2O-N kg^{-1} day^{-1}) denitrification potential [mean denitrification potential of water column (0.72 mg N_2O-N kg^{-1} day^{-1}) plus mean rhizosphere denitrification potential (96 mg N_2O-N kg^{-1} day^{-1})] was lower. Therefore, it is likely that the rest of NO_3^--N would be removed by other pathways contributing to removal of NO_3^--N. The major NO_3^--N removal pathways are plant and microbial

assimilation and sediment and/or soil denitrification. Rhizosphere denitrification potential in this study was observed to significantly contribute towards NO_3^--N removal.

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BIOGRAPHICAL SKETCH

Bhuvaneswari Govindarajan, shortly called as Bhuvana, was born in 1976 in India. She spent the greater portion of her life growing up in a small town, named Vandavasi, Tamilnadu, India. Hailing from a farming family, she was fascinated by field of agriculture. She majored in horticulture sciences and received her Bachelor's degree in Horticulture (1994-1998) from Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India. She completed her Master of Science in Vegetable Crops (1999-2001), with a minor in plant breeding, from Punjab Agricultural University, Ludhiana, Punjab, India.

For the next four years, she worked in several organizations, as research associate in a private biofertilizer production unit, as a lecturer in agricultural college, as a technical consultant for an MNC, on National Horticulture Board project, and as a Development Executive for an NGO in a watershed development project. While working in an NGO, she was exposed to problems associated with environmental degradation/pollution and inclined towards the field of environmental science. She then decided to continue her education in the field of environmental sciences. Upon achieving the graduate research assistantship, she came to United States, to pursue her Masters degree in Interdisciplinary Ecology at University of Florida. Bhuvana has had an opportunity to work on a project as well as her thesis research focusing on nutrient removal from nonpoint source pollution using constructed wetlands.

After gaining hands on experience in the US, She wish to return to her county, India, where her knowledge and skills will be applied towards achieving cleaner, greener, and sustainable environment.