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$CBOD_5$ treatment and nitrogen transformations of the marshland upwelling system in intermediate and saltwater marshes

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$CBOD_5$ TREATMENT AND NITROGEN TRANSFORMATIONS OF THE MARSHLAND UPWELLING SYSTEM IN INTERMEDIATE AND SALTWATER MARSHES

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DEDICATION

This research is dedicated to the memory of Robert E. Watson, Jr., the contributor of the Moss Point data, who passed away May 3, 2002, before his research was completed.

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ABSTRACT

The marshland upwelling system (MUS) was designed to treat domestic wastewater from coastal dwellings where conventional methods are inadequate due to high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater. Currently there is no adequate treatment system available and coastal dwellings are contributing to water quality problems. This study focused on determining the treatment effectiveness of the MUS for organic matter and understanding the specific processes involved in nitrogen treatment.

The treatment of organic matter, measured as five-day carbonaceous biological oxygen demand ($CBOD_5$), was effective in field tests for both saltwater and intermediate marshes. Global removal efficiencies were 95 and 99% and first-order removal constants were 0.80 and 1.30 m^{-1} for saltwater and intermediate marshes, respectively. A laboratory study confirmed effective treatment of organic matter.

Wastewater nitrogen present as nitrate was removed very effectively (> 99%) during a laboratory simulation study. Ammonium removal was not as effective and dropped to 20% by the conclusion of this study. Removal of ammonium was dependent upon the sorption capacity of the soil under reducing conditions. While a field site would offer a much greater soil treatment volume and longer effective retention, ammonium would eventually saturate the soil sorption capacity which will be important in determining MUS longevity. As nitrogen is primarily present as ammonium in the wastewater, conversion of ammonium to nitrate, via nitrification, prior to injection would improve performance. A preliminary oxidation study yielded a 20% conversion of ammonium to nitrate. Further research should focus on improving nitrification rates prior to injection.

Additionally, a higher salinity (20‰, relative to 2‰) was found to have an initial, significantly negative impact on treatment of carbon, sorption of ammonium, and denitrification.

Therefore, the impact of the native salinity should be considered in any future design modifications to the MUS.

In conclusion, the MUS has demonstrated great effectiveness in treating wastewater carbon and nitrate. This research showed additional work is required to enhance nitrification rates prior to injection to improve ammonium treatment. Thus, with modifications, the MUS stands to become the first, long-term, effective treatment system for coastal dwelling wastewater.

CHAPTER 1: GLOBAL INTRODUCTION

1.1 Introduction

Wetlands were once considered to have very little or no value to human society, but are now recognized for the many functions and services they provide. They serve as habitat and protection for a number of fish and wildlife species; as sources, sinks and transformers of many biological and chemical resources; as buffering from storm surges and mitigation of flooding; to recharge and discharge groundwater; and to stabilize sediment and prevent erosion (Patrick, 1994; Woodward and Wui, 2001). Wetlands have the ability to remove nutrients and contaminants from surface waters, a function of growing importance as pollutants from anthropological sources increase. Because of these properties, natural wetlands have long been used for waste treatment purposes. Peoples of ancient China and Egypt filtered their wastes through natural wetlands. More recently, in the 1960s and 70s, researchers have realized the potential benefits natural and constructed wetlands provide as waste treatment systems (Kadlec and Knight, 1996; Mitsch and Gosselink, 2000). In contrast to constructed wetlands, natural wetlands used as waste treatment systems have the benefit of being a cheap and effective alternative to many other waste treatment systems (Kadlec and Knight, 1996). Fewer studies exist on natural wetlands used for wastewater treatment because more regulations exist on the uses of natural wetlands. However, the abundance of wetlands in Louisiana often makes it unnecessary to build constructed wetlands (Cardoch et al., 2000). Costs of building constructed wetlands for wastewater treatment are often eliminated in coastal Louisiana as the wetlands are already in place, further decreasing startup costs and increasing savings. Day, et al., (2004) estimated that three natural Louisiana wetlands, treating wastewater from two municipalities and a shrimp processing facility, provided a capitalized cost savings ranging from $500,000 – $2,636,000 over a 20-year period.

Pollutants generally associated with wastewaters include suspended solids, biochemical and chemical oxygen demand, pathogens, metals, and nutrients. One of the most common measurements used as a proxy for organic matter is five-day carbonaceous biochemical oxygen demand ($CBOD_5$). Carbonaceous BOD_5 is often used in determining the effectiveness of many waste treatment systems, including subsurface wetlands (Kadlec and Knight, 1996; Nguyen, 2000; Vymazal, 2002; Al-Omari and Fayyad, 2003; Mantovi et al., 2003; Metcalf and Eddy, 2003; Meuleman et al., 2003; Akratos and Tsihrintzis, 2007; Aslam et al., 2007; Jou et al., 2009). Carbonaceous BOD reduction in wastewater is achieved by several physical, chemical, and biological processes, including filtration and decomposition (Gopal, 1999; Meuleman et al., 2003). Decomposition of organic matter occurs more rapidly under aerobic conditions than the anaerobic conditions found in wetland subsurface soils (D'Angelo and Reddy, 1999; White and Reddy, 2001). However, wetland soils retard the movement of water and, subsequently, soluble organic matter flowing through the wetland subsurface has a significantly longer retention time than is generally found in open water. The longer retention time provides extended exposure of the organic matter to the microorganism consortium performing the decomposition processes in the subsurface sediments.

Nutrient discharge is of concern when concentrations are excessive, which can lead to eutrophication. However, the level of particular nutrients required to cause eutrophication varies (Camargo and Alonso, 2006; Burkholder et al., 2007). Some ecosystems are more nutrient limited than others and, thus, conditions necessary to bring about eutrophication vary. In addition to eutrophication, nitrogen, in certain forms, can be toxic to some aquatic species (Kadlec and Knight, 1996; Camargo and Alonso, 2006; Paerl, 2006; Burkholder et al., 2007). The marshland upwelling system (MUS) was designed to treat domestic wastewater in coastal wetland environments. One of the primary goals of the MUS is removal of phosphorus and

nitrogen associated with the wastewater (Fontenot et al., 2006; Evans and Rusch, 2007a, b). Nitrogen, in its various forms, can undergo several different microbial transformations. However, the end products of these transformations vary based on the environment in which they occur. Certain transformations can lead to loss of nitrogen to the atmosphere as dinitrogen gas (N_2) or nitrous oxide (N_2O), thus permanently removing nitrogen from the system. Permanent removal of nitrogen from the system would be the most optimal scenario; however, N_2O is a potent greenhouse gas and releasing significant amounts to the atmosphere would be undesirable.

Net negative charges of clay and organic matter present in the soil attract and adsorb NH_4^+ as opposed to NO_3^-, which is repelled. Because NH_4^+ is the main form of nitrogen being injected by the MUS, there is some concern that, over time, the soil may become saturated with NH_4^+ and, therefore, reduce the ability of the system to remove nitrogen. Though the negative charges of the soil attract NH_4^+, other abundant cations in coastal ecosystems, such as K^+, Na^+, Fe^{2+}, Ca^{2+}, and Mg^{2+} are also attracted and compete with NH_4^+ for sorption sites. The degree to which the soil retains NH_4^+ over other cations plays an important role in how soon the soil will become saturated with respect to NH_4^+. Conditions within the MUS are conducive to retention of NH_4^+ through sorption to soil (until saturation is reached) because, other than anammox, there is no process which permanently removes NH_4^+ from the system. Anammox requires oxidation by NO_2^- (Megonigal et al., 2004) and under present conditions, very little NO_2^- is being injected. For long-term success of the MUS there must be an efficient method for permanent nitrogen removal by transformation to N_2O or N_2. Nitrification of NH_4^+ to NO_3^- before injection *may* lead to increased denitrification and loss of nitrogen via N_2. However, higher salinities are known to increase physiological stress in microbes, which could lead to a decrease in microbial diversity (de Franca et al., 2000; Yoshie et al., 2004; Grommen et al., 2005). Yoshie, et al., (2004) has shown that salinities near that of seawater decrease the nitrite reductase gene diversity in

wastewater treatment systems. This loss of gene diversity may lead to a decrease in the ability of the subsurface environment to fully convert NO_2^- to N_2. It is imperative that NO_2^- be further reduced in order to form N_2 and remove nitrogen from the system.

1.2 Project Objectives

The main questions of this study are:

1) What is the effectiveness of the MUS in removing organic matter from the wastewater as measured by $CBOD_5$?
2) What nitrogen transformations are occurring, at what rate, and what is the fate of the forms generated within the MUS?
3) Will an oxidizing treatment in the primary collection tank convert substantial amounts of ammonium to nitrate prior to injection?
4) Can denitrification of wastewater nitrate be significantly enhanced and, thus, lead to removal of nitrogen from the system?
5) Does a more saline environment significantly impact the treatment of $CBOD_5$ and the rate of nitrogen removal?

Questions 1 and 5 were addressed in a field study, while questions 2 – 5 were addressed in a laboratory study.

CHAPTER 2: LITERATURE REVIEW

2.1 Carbon

2.1.1 Introduction

Carbon is the basic building block of life. In wetlands, carbon is stored in vegetation, fauna, microbial biomass, plant litter, and soil organic matter (DeBusk et al., 2001). Because wetlands generally have high rates of primary production and low rates of decomposition, they often serve as net sinks for carbon (Schlesinger, 1997). Storage of organic carbon in wetland soils is important in the global carbon cycle (Happell and Chanton, 1993; Whiting, 1994). However, wetlands have been attributed to be a source of the potent greenhouse gas methane to the atmosphere (Yu et al., 2006; Reddy and DeLaune, 2008), especially in freshwater wetlands, which typically have a higher methane emission than saltwater wetlands (DeBusk et al., 2001; Chmura et al., 2003). An understanding of the processes that drive carbon cycling is critical, especially as carbon plays a significant role in global warming.

2.1.2 Cycling

Atmospheric carbon is converted from inorganic carbon (CO_2) to organic carbon through photosynthesis by standing vegetation as well as algae. As plants die off, they become detritus, which then undergoes decomposition (Figure 2.1). The detritus created by plants becomes the main source of carbon in most wetlands (Reddy and DeLaune, 2008). Labile fractions of the detritus are decomposed to inorganic constituents, while the more recalcitrant fractions are accreted and form new peat layers or soil humus. Labile fractions are converted to inorganic constituents by abiotic leaching and fragmentation into smaller pieces by meiofauna, extracellular enzyme hydrolysis of nucleic acids, cellulose, and proteins into monomers, and aerobic and anaerobic consumption by heterotrophic microorganisms (DeBusk et al., 2001). Extracellular enzymes, excreted by fungi and bacteria, hydrolyze biopolymers into oligomers

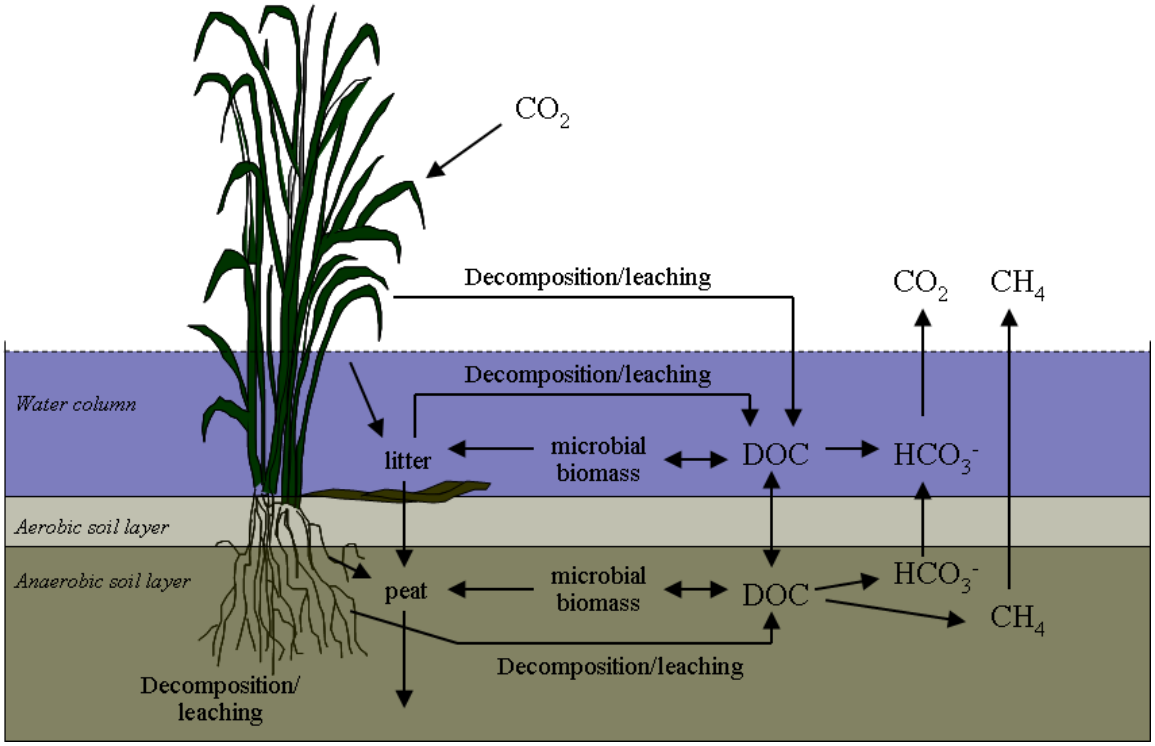


Figure 2.1 Carbon cycling in wetlands. Adapted from DeBusk, et al., (2001).

and monomers that can then be utilized by microorganisms. In organic matter decomposition, enzyme hydrolysis is generally considered to be the rate-limiting step (Sinsabaugh et al., 1993). Activities of some enzymes, such as glucosidase, protease, and phosphatases, were found to decrease with more reducing conditions (McLatchey and Reddy, 1998), so as redox potentials decrease, microbial growth and activity also decreases.

Once the high molecular weight compounds have been broken down to low molecular weight compounds via enzyme hydrolysis, heterotrophic microorganisms utilize the low molecular weight compounds as carbon and energy sources. The organisms involved depend on the availability of electron acceptors, such as O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, and CO_2, for respiration. Microorganisms couple oxidation of organic carbon (electron donors) with the reduction of electron acceptors to obtain the energy necessary for growth in the form of ATP (DeBusk et al., 2001; Reddy and DeLaune, 2008). Most wetlands contain several electron acceptors; therefore, competition exists between microbial groups. Those organisms that derive

the most energy, and have the fastest degradation kinetics, outcompete other groups (DeBusk et al., 2001). Aerobic bacteria will outcompete anaerobic bacteria, while anaerobic bacteria generally utilize electron acceptors in the order that yields the most energy, or NO_3^- > Mn^{4+} > Fe^{3+} > SO_4^{2-} > CO_2 (Reddy and DeLaune, 2008). The stratification in microbial activity and electron acceptors that is often observed in wetland profiles is explained by this phenomenon (Reddy and D'Angelo, 1994).

2.1.2.1 Organic Matter

Organic matter is a mixture of plant, microbial, and animal sources in various stages of decomposition and can be grouped as nonhumic substances (carbohydrates, proteins, and fats), phenolic substances (lignins and tannins), and humic substances (heterogeneous mixtures of high molecular weight aromatic structures) (Stevenson, 1994). Both external and internal sources contribute to the organic matter added to wetlands. Internal sources include plant matter, litter fall, and detrital matter. External sources include both point and nonpoint sources within the watershed. Point sources can consist of organic matter loads from sewage effluents, for example, which are generally only added when wetlands are used as a means of treating the wastewater (Reddy and DeLaune, 2008).

The lower energy yield of anaerobic processes versus aerobic processes leads to slower microbial decomposition rates and higher organic matter accumulation seen in wetlands versus upland systems (DeBusk and Reddy, 1998). The low decomposition rates lead to a significant accumulation of organic matter that is moderately decomposable, in addition to recalcitrant fractions (Clymo, 1983). In wetland ecosystems, organic matter accumulation is the primary source of potential energy to microbial communities. It regulates long-term storage of compounds, such as nutrients, heavy metals, and organics, and is a major component in the global carbon cycle as a source/sink for greenhouse gases (Reddy and DeLaune, 2008).

The decomposition of organic matter is achieved through the activity of microbial communities, which are in turn influenced by environmental conditions. The microbial decomposer community responds to substrate quality, physicochemical environment (includes temperature, pH, exogenous nutrient supply, and electron acceptor availability), and other organisms (Swift et al., 1979; Heal et al., 1981; Reddy and D'Angelo, 1994; DeBusk et al., 2001). Decomposition rates are limited by microbial growth, which is affected, in part, by the availability of nutrients. Most often in wetlands, N and P are limiting (Westermann, 1993). Depletion of oxygen forces a shift in microbial metabolism from aerobic to anaerobic pathways, thus slowing microbial activity. Sulfate reducing and methanogenic bacteria are also dependent on fermenting bacteria to produce short-chain carbon compounds, which they require for growth as they cannot utilize high molecular weight compounds (Howarth, 1993).

Biochemical oxygen demand, or BOD, is a measurement of the dissolved oxygen used in the biologically mediated oxidation of organic matter by microorganisms. Biochemical oxidation is assumed to be proportional to the amount of biodegradable organic matter remaining in a sample. Typically, the procedure is carried out over a five day period and is subsequently called five-day BOD, or BOD_5. Five-day carbonaceous biochemical oxygen demand, ($CBOD_5$) differs from BOD_5 in that nitrification is inhibited. Nitrification causes the measured BOD value to be higher due to the oxidation of ammonium by nitrifying bacteria. Carbonaceous BOD measures the oxygen demand created solely by the oxidizable carbon in the sample. The use of BOD as a measurement of organic matter in wastewater is prevalent because BOD test results: 1) allow for the determination of the approximate quantity of oxygen required to biologically stabilize the organic matter present, 2) are used in the determination of waste-treatment facility sizes, 3) are used to measure the efficiency of some treatment processes, and 4) help determine compliance of wastewater discharge permits (Metcalf and Eddy, 2003).

2.1.2.2 Microbial Biomass

In wetlands, the majority of the organic carbon fixed (by phytoplankton and macrophytes) is processed and recycled entirely by bacteria without ever entering the foodweb (Wetzel, 1984). Organic matter decomposition is the primary ecological role of heterotrophic microorganisms in soils (Reddy and DeLaune, 2008). Though the microbial biomass is a small fraction of the organic matter, most of the net ecosystem production passes through the microbial component at least once and typically several times (Elliott et al., 1984; Heal and Ineson, 1984; Van Veen et al., 1984). The microbes derive their energy and carbon for growth from the soil organic matter and mineralize growth-limiting nutrients and form recalcitrant organic compounds (DeBusk et al., 2001). Thus, soil microbes can exert a significant influence on the energy flow of a wetland ecosystem through feedback, because mineralization of organically bound nutrients regulates nutrient availability for both primary production and decomposition (Elliott et al., 1984).

Some labile organic compounds are hydrolyzed by enzymes and assimilated into microbial biomass, leaving the remaining to be oxidized to CO_2. Though the microbial biomass represents only a small fraction of soil organic carbon (approximately 3 – 5% of the total), it is responsible for increasing the refractory portions of the organic carbon pool as more labile components are mineralized to CO_2 or assimilated into cell biomass. Thus, the soil microbial biomass constitutes a significant carbon sink, as it represents a large portion of the active organic carbon pool. Turnover of soil organic matter may be several years, whereas turnover of the active biomass may be on the order of days (Reddy and DeLaune, 2008).

2.1.2.3 Methanogenesis

The electron acceptor that yields the least amount of energy is CO_2. Methanogenic bacteria reduce CO_2 to CH_4 utilizing three main substrates, CO_2-type substrates (CO_2, CO, and

formate), methyl substrates (methanol, methylamine, dimethylamine, trimethylamine, methylmercaptan, and dimethylsulfide), and acetate (Madigan et al., 2006). Methanogens are obligate anaerobes that grow heterotrophically and autotrophically. Autotrophic methanogens use CO_2 as an electron acceptor and a carbon source and H_2 as the primary electron donor. Heterotrophic methanogens use acetate or other organic carbons directly for growth and also as an energy source (Reddy and DeLaune, 2008). Sulfate-reducing bacteria are more effective competitors for the same compounds (i.e. acetate and H_2) than methanogens and, thus, in most environments, there is little overlap between sulfate reduction and methanogenesis (Schlesinger, 1997). Methanogenesis in marine environments is generally inhibited by the high amounts of sulfate available and, therefore, is more prevalent in freshwater than saltwater wetlands (DeBusk et al., 2001).

Wetland methane fluxes account for a large portion of the total global methane flux to the atmosphere (Schlesinger, 1997). Methane loss to the atmosphere is highest when dissolved methane concentrations in the soil exceeds the hydrostatic pressure of the overlying waters, which allows bubbles of gas to form and escape to the surface via ebullition (Schlesinger, 1997). Ebullition can account for a large fraction of the methane flux to the atmosphere (Wilson et al., 1989); however, wetland vegetation can act as conduits for the escape of methane to the atmosphere (Sebacher et al., 1985).

2.2 Nitrogen

2.2.1 Introduction

Nitrogen is generally a limiting nutrient because of the many transformations that it undergoes. At any given time, the majority of nitrogen in a wetland ecosystem is incorporated into plant and microbial living biomass, detrital matter, precipitated or sorbed onto soil particles as humic materials, or buried by sedimentation and detrital accumulation. Nitrogen is

continually lost from the system as gaseous products, which decreases the pool of nitrogen available for plants and microorganisms to utilize for growth. If there is not a continual input of nitrogen from an external source, even with the turnover that occurs, nitrogen will eventually be lost from the system. Nitrogen is especially limiting in northern peatland systems, such as bogs, fens, tundra, and boreal forests, because nitrogen inputs are minimal and temperatures are colder, which reduces mineralization to nutrient forms (Schlesinger, 1997). A comprehensive understanding of the various processes that affect nitrogen cycling in wetland ecosystems is imperative for the development of a more globally accurate perspective.

2.2.2 Cycling

Nitrogen cycling in wetland soils is supported by the juxtaposition of aerobic and anaerobic zones (Figure 2.2). Various transformations occur in both zones as soluble organic and inorganic nitrogen forms diffuse across the boundary between aerobic and anaerobic zones. Nitrogen fixation is an important source of inorganic nitrogen. Organic nitrogen is stored in submerged and emergent vegetation, fauna, standing dead and detrital plant material, peat, soil organic matter, and the microbial biomass. Heterotrophic microorganisms convert organic nitrogen to ammonium (NH_4^+) via ammonification, a mineralization pathway, which can occur under both aerobic and anaerobic conditions (Schlesinger, 1997; DeBusk et al., 2001). Inorganic nitrogen exists as NH_4^+, nitrate (NO_3^-), and nitrite (NO_2^-). Various processes affect the concentration of NH_4^+, including plant uptake, immobilization by microbes, and sorption to clay minerals. Ammonium can also be lost via volatilization, in which NH_4^+ is converted to its gaseous form, NH_3, at high pH values. The obligate, two-step, aerobic process of nitrification converts NH_4^+ to NO_3^- (Schlesinger, 1997). As NO_3^- diffuses into the anaerobic layer, it undergoes denitrification. Nitrate can also be converted to NH_4^+ via dissimilatory nitrate reduction to ammonium (DNRA).

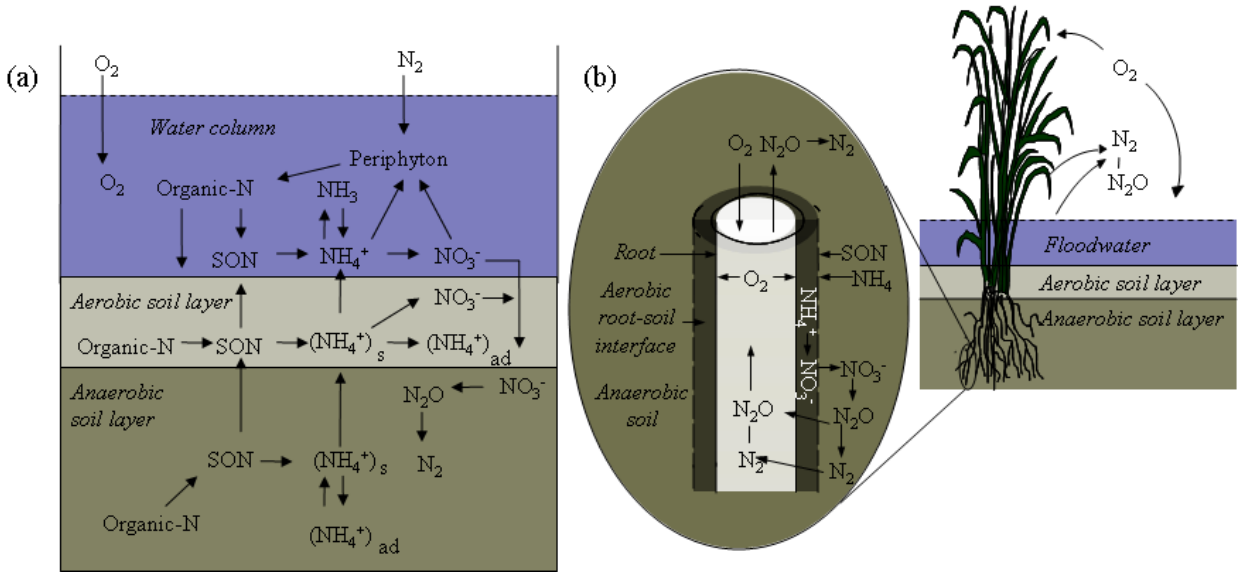


Figure 2.2 Wetland nitrogen cycle showing aerobic-anaerobic regions (a) at the soil/water interface and (b) at the root/soil interface. Adapted from DeBusk, et al., (2001).

2.2.2.1 Fixation

Biological fixation of nitrogen occurs in the water column, aerobic soil-floodwater interface, root zone, and some anaerobic portions of the soil. It is the only biological process that converts the inert gas, N_2, to organic and inorganic nitrogen forms that are biologically available (DeBusk et al., 2001). Only a limited number of prokaryotic organisms can fix N_2 in a process driven by the nitrogenase enzyme. Several genera of bacteria and cyanobacteria perform the process and are commonly found in wetlands (Buresh et al., 1980; Howarth et al., 1988b; Vymazal and Richardson, 1995). Fixation is positively influenced by low N:P ratio inputs, reducing conditions, molybdenum (Howarth and Cole, 1985), iron (Paerl et al., 1994), and dissolved organic matter (Howarth et al., 1988a) and negatively influenced or inhibited by the presence of oxygen, sulfate, and dissolved inorganic nitrogen (Howarth et al., 1988a; DeBusk et al., 2001). Though total nitrogen levels are generally high in wetlands, the slow mineralization of organic nitrogen, under anaerobic conditions, to nutrient forms of inorganic nitrogen contributes to nitrogen limitation for plant growth. Therefore, fixation can provide an important source of additional nutrient nitrogen and contribute to plant productivity.

2.2.2.2 Plant Uptake and Influences on Nitrogen Cycling

Vegetation plays a significant role in wetland nitrogen cycling. Wetland plants assimilate inorganic nitrogen forms (NH_4^+ and NO_3^-) into plant tissue and thereby immobilize it from further cycling within the system until the plants die and decay. Plants are also responsible for the rhizosphere, a very important feature in wetlands. The rhizosphere is the area directly adjacent to plant roots in which some oxygen is present due to diffusion from the atmosphere to the soil via transport through the plant. The plants, therefore, provide an additional source of oxygen to the generally anaerobic soil layer (Mitsch and Gosselink, 2000; Reddy and DeLaune, 2008). The rhizosphere provides an environment in which nitrification and denitrification are coupled and additional nitrogen cycling can occur. Because of the close proximity of aerobic and anaerobic soil environments in the rhizosphere, efficiency of nitrification/denitrification coupling is increased. Ammonium, produced by mineralization, diffuses into the aerobic rhizosphere due to existing concentration gradients, and is nitrified to NO_3^-. Nitrate then diffuses into the anaerobic soil near the plant root, also due to concentration gradients, and is denitrified to N_2 gas, which then diffuses out of the soil and back into the atmosphere, removing it from the wetland subsurface (DeBusk et al., 2001; Reddy and DeLaune, 2008).

Efficiency of nitrogen utilization (here defined as the "increase in plant nitrogen per unit mass of available nitrogen") by vegetation is highly variable and depends on the type of wetland (herbaceous vs. forested) in which the plants grow (DeBusk et al., 2001). Forested wetland litter generally decomposes slower than litter from herbaceous wetlands, which slows the rate of nitrogen turnover in forested wetlands (DeBusk and Reddy, 1987). It has been shown that nitrogen use efficiency of wetland vegetation and the C:N ratio of plant litter decreased with nutrient loading (Shaver and Melillo, 1984; Reddy and Portier, 1987; Koch and Reddy, 1992). Plant use of nitrogen has been shown to increase with increasing temperature. Temperature

increases from 10 to 25°C have been shown to increase nitrogen use efficiency from 5 to 38% in *Typha* spp. (Reddy and Portier, 1987). The majority of the nitrogen plants use is from the soil porewater, where they compete directly with soil microorganisms. Plants, however, are very important for microbial populations. Once plants die, microorganisms begin to break down their detrital material, which provides important nutrients for microbial growth. Additionally, in wetlands where the soil environment is largely anaerobic and inorganic nitrogen is in small supply, decaying plant material is an important source of inorganic nitrogen (DeBusk et al., 2001).

2.2.2.3 Mineralization/ammonification

Nitrogen is converted from organic nitrogen to inorganic nitrogen via mineralization. The primary pathway for mineralization of organic nitrogen in wetlands is ammonification. This process, which is performed by a wide variety of heterotrophic microorganisms, converts organic nitrogen to NH_4^+. Ammonification occurs under both aerobic and anaerobic conditions, but more slowly under anaerobic conditions. The complex organic nitrogen compounds present in the soil are hydrolyzed by extracellular enzymes into simple monomers (Gardner et al., 1989; McLatchey and Reddy, 1998; DeBusk et al., 2001). A wide range of bacteria, in addition to some fungi and plant roots of many wetland species, synthesize these enzymes, which have been reported to retain their activity for up to a year (McLatchey and Reddy, 1998). These enzymes eventually break down the organic nitrogen into simpler compounds, followed by a breakdown of the amino acids that subsequently results in the liberation of NH_4^+ (Gardner et al., 1989; DeBusk et al., 2001). Ammonification is generally limited by the hydrolysis rate of the more complex, large organic compounds (Stanford and Smith, 1972).

Under anaerobic conditions, NH_4^+ levels increase due to the lower nitrogen requirements of anaerobic microorganisms (DeBusk et al., 2001). The restricted supply of oxygen leads to the

use of alternative electron acceptors, which influences the microbial catabolic processes mediating the rate of organic matter decomposition in wetland soils (D'Angelo and Reddy, 1999). The microbial pool is the single greatest regulator of organic nitrogen stability and, thus, plays a large role in regulating nutrient release, which affects wetland surface water quality (DeBusk et al., 2001).

2.2.2.4 Ammonium Sorption

Ammonium can be sorbed onto wetland soils and organic matter associated with soils. There are two types of NH_4^+ sorption to clay minerals: an ion exchange reaction that occurs on the surface of negatively charged clays (or exchangeable NH_4^+) and sorption into the interlayers of the clay structure (or fixed NH_4^+), exhibited by some types of clay minerals. Ammonium also sorbs to organic matter due to the natural negative charge organic matter carries. Generally the higher the organic matter content, the higher the NH_4^+ sorption (Rosenfeld, 1979; Hou et al., 2003; Fernando et al., 2005). Ammonium sorption is also influenced by pH, salinity, temperature, cation exchange capacity (CEC), and ionic strength (Dalal, 1975; Gardner et al., 1991; Seitzinger et al., 1991; Evangelou, 1998; Rysgaard et al., 1999; Demir et al., 2002; Hou et al., 2003). As the pH increases, NH_4^+ changes form to NH_3, which volatilizes and, therefore, decreases the amount of NH_4^+ available for sorption. For optimum NH_4^+ sorption, the pH should be below 7 (Evangelou, 1998; Demir et al., 2002). An increase in temperature will increase the exchange capacity of a soil as ion exchange is an endothermic process. Thus, an increase in temperature will increase NH_4^+ sorption (Demir et al., 2002). Salinity (i.e., ionic strength) has a profound effect on NH_4^+ sorption, which decreases with increased salinity (Gardner et al., 1991; Seitzinger et al., 1991; Rysgaard et al., 1999; Hou et al., 2003). There is also an increased NH_4^+ desorption with increasing salinity (Rysgaard et al., 1999). This is partially due to increased competition for sorption sites with other cations as salinity is increased. Anions present in

seawater also partially neutralize the charge of NH_4^+ ions, as anions form ion pairs with NH_4^+, and thus reduce the sorption of NH_4^+ to soil particles (Dalal, 1975; Gardner et al., 1991; Rysgaard et al., 1999). An anion that is strongly sorbed by soil (i.e., PO_4^{3-}) may increase the sorption of its associated cation (in this case, NH_4^+). However, irrespective of the anion present, sorption increases with increasing CEC (Dalal, 1975).

2.2.2.5 Volatilization

Nitrogen is lost as NH_4^+ from wetland soils and overlying floodwaters via volatilization, when NH_4^+ is converted to NH_3 (Equation 2.1).

$$NH_4^+ + OH^- \leftrightarrow NH_3(g) + H_2O \quad (2.1)$$

This abiotic process is controlled by the pH of the soil-water interface and is not significant below pH 7.5. In wetlands, a pH of 7.5 is only encountered in the overlying water body due to the diurnal changes caused by photosynthesis and respiration (DeBusk et al., 2001). Photosynthesis and respiration (molar based, Equation 2.2) (Redfield et al., 1963) in the overlying water column regulate the pH and can sometimes cause a swing of 2-3 pH units.

$$\text{Photosynthesis} \rightarrow$$
$$106CO_2 + 16HNO_3 + H_3PO_4 + 122H_2O \leftrightarrow (CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 138O_2 \quad (2.2)$$
$$\leftarrow \text{Respiration}$$

The CO_2 consumed and created by photosynthesis and respiration, respectively, is a key component to changes in pH. Carbon dioxide interacts with water according to the equations in the bicarbonate-carbonate system, which influences the pH as seen in Equation 2.3.

$$CO_2\,(aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-}. \quad (2.3)$$

Diurnal fluctuations in pH allow the pH to rise (typically during the day), which allows for ammonia volatilization and subsequent loss of NH_4^+ from the water column (Reddy and Patrick,

1984). Increases in the pH in the range of 8.5 to 10 will dramatically increase the rate of volatilization (Reddy and Patrick, 1984).

2.2.2.6 Nitrification

Ammonium is converted to NO_3^- via the obligate aerobic process, nitrification. The first step (Equation 2.4) oxidizes NH_4^+ to NO_2^-, while the second step (Equation 2.5) converts NO_2^- to NO_3^-.

$$2\ NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \quad (2.4)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2.5)$$

The overall equation (on a mass basis), including cell synthesis is shown in Equation 2.6.

$$NH_4^+ + 1.815O_2 + 0.1304CO_2 \rightarrow 0.0261C_5H_7O_2N + 0.973\ NO_3^- + 0.921H_2O + 1.973\ H^+ \quad (2.6)$$

Autotrophic bacteria couple the oxidation of NH_4^+ to electron transport phosphorylation while utilizing inorganic carbon to synthesize cellular components required for microbial growth (DeBusk et al., 2001). The oxidation state of nitrogen is increased from -3 for NH_4^+ to +3 for NO_2^- or +5 for NO_3^-.

Nitrification occurs in the water column, aerobic soil layer, and aerobic portions of the rhizosphere. The availability of oxygen, NO_2^-, alkalinity, and NH_4^+ are the main limiting factors of nitrification. In the majority of wetlands, NH_4^+ is supplied to aerobic layers through diffusion from anaerobic soil layers due to a concentration gradient between the two layers. Alkalinity, pH, temperature, and salinity all play a key role in regulating nitrification. Optimal temperature ranges from 30 to 40°C (Reddy and Patrick, 1984). The optimal pH range for nitrification is near 8.0, while rates decline significantly below pH 6.8 (Simek and Cooper, 2002; Metcalf and Eddy, 2003). It has been shown that nitrification can occur in acid soils; however, the reasons for this are currently unclear. One possible explanation may be the lack of exchangeable base-forming cations, which has been shown to decrease nitrification rates in acid mineral and peat

soils (Brady, 1990; Simek and Cooper, 2002). Copper, calcium, magnesium, molybdenum, nickel, and zinc are important micronutrients for nitrifying microbial communities; however, in high enough concentrations copper and nickel can be toxic along with chromium. High levels of NH_3 can also inhibit nitrifiers (Metcalf and Eddy, 2003). Various researchers have found that at increased salinity there is a decrease in the nitrification activity (Gardner et al., 1991; Seitzinger et al., 1991; Stehr et al., 1995; Rysgaard et al., 1999; Bollmann and Laanbroek, 2002). The negative correlation between nitrification and salinity has been attributed to increased ammonia efflux from the sediment (Seitzinger et al., 1991; Rysgaard et al., 1999), ion pairing between NH_4^+ and seawater anions reducing the availability of NH_4^+ (Gardner et al., 1991), changes in the community composition of ammonia oxidizers induced by salinity (Bollmann and Laanbroek, 2002; Coci et al., 2005), or adverse effects of salinity on microbial metabolism (Stehr et al., 1995; Rysgaard et al., 1999; Bernhard et al., 2007).

The first step of nitrification is ammonia oxidation (Coci et al., 2005) and the bacteria that perform this step are collectively called ammonia-oxidizing bacteria (AOB) and are lithoautotrophic (Koops and Pommerening-Roser, 2001). The second step of nitrification, nitrite oxidation, is also performed by lithoautotrophic bacteria (Koops and Pommerening-Roser, 2001), commonly referred to as nitrite oxidizing bacteria (NOB). Known AOB are represented by two phylogenetically distinct groups, one located within the γ subclass and the second by the β subclass, both of the Proteobacteria. Within the γ subclass only one genus, *Nitrosococcus*, has been defined. The β subclass is represented by two genera, *Nitrosospira and Nitrosomonas*, with *Nitrosomonas* having the highest number of species (Koops and Pommerening-Roser, 2001). *Nitrosomonas* and *Nitrosospira* have been found in a wetland in the Netherlands (Kowalchuk et al., 1998); however, this is one of the few studies that attempt to describe the AOB microbial community in a wetland. Known NOB are represented by four phylogenetically distinct groups,

three of which belong to subclasses of the Proteobacteria, α, γ and δ, while the fourth belongs to a distinct phylum most closely related to the δ subclass. The genus *Nitrobacter* belongs to the α subclass, *Nitrococcus* to the γ subclass, and *Nitrospina* to the δ subclass. Species from the genus *Nitrospira* belong to the fourth group (Koops and Pommerening-Roser, 2001).

Ammonia monooxygenase is the catalyzing enzyme of ammonia oxidation and is coded by the gene *amoA* (Francis et al., 2005), which is used in genetic studies of the AOB community. It has been hypothesized that oxygen levels play a key role in regulating changes of phylogenetic clustering in microbial communities. However, Kowalchuk et al., (1998) demonstrated that no single phylogenetic cluster, in a microbial community associated with the rhizosphere of *Glyceria maxima*, a wetland plant, was specific to environments that are periodically anoxic. The enzyme which catalyzes the second step of nitrification is nitrite oxidoreductase and is coded by the gene *nxrA*. Few studies have been found that discuss the genetic influences of the *nxrA* gene as analysis utilizing the gene has just recently been developed (Poly et al., 2008).

2.2.2.7 Denitrification

Nitrogenous oxides are reduced to N_2 via the microbial-mediated process of denitrification (Equation 2.7) (Knowles, 1982; Schlesinger, 1997).

$$5CH_2O + 4H^+ + 4NO_3^- \rightarrow 2N_2 + 5CO_2 + 7H_2O \quad (2.7)$$

The cell synthesis (on a mass basis) with methanol as an electron donor is shown in Equation 2.8.

$$0.1667CH_3OH + 0.1561NO_3^- + 0.1561H^+ \rightarrow$$

$$0.00954C_5H_7O_2N + 0.0733\ N_2 + 0.3781H_2O + 0.119CO_2 \quad (2.8)$$

Denitrifiers are facultatively anaerobic, although denitrification only requires anoxic environments (Schlesinger, 1997). Denitrification occurs through a stepwise reduction during which nitrogen changes oxidation states four times (NO_3^- (+5) → NO_2^- (+3) → NO (+2) → N_2O

$(+1) \rightarrow N_2(0)$). Denitrification is the major removal process of nitrogen in wetlands because of the lack of available oxygen, indicated by a low redox potential (E_h), and the generally high organic carbon content prevalent in most wetland soils. When oxygen is unavailable, denitrifiers will begin using NO_3^- as an alternative terminal electron acceptor. Denitrification generally occurs between E_h levels of 200 and 300 mV (Reddy and DeLaune, 2008). The redox status of wetland soils can control the extent to which denitrification is able to occur within the soil profile, because denitrifiers will use oxygen if present (Yu and Patrick, 2004). Therefore, denitrification is not likely to occur in a soil until all oxygen has been consumed. Nitrate is often provided by diffusion from the overlying water column, the thin oxygenated surficial soil, or the rhizosphere due to the process of nitrification. Wetland soils commonly have conditions that are more conducive to denitrification than upland soils, such as a higher organic carbon content and lower oxygen status. These conditions are due to the poor drainage and presence of floodwaters that are indicative of wetlands (DeBusk et al., 2001). Denitrification is the primary process of inorganic nitrogen removal from wetland ecosystems. Because of these capabilities, wetlands often serve to significantly enhance water quality (DeBusk et al., 2001).

The usually high organic carbon content of wetland soils generally provides abundant substrate for the heterotrophic denitrifying bacteria, so that NO_3^- becomes the limiting factor in denitrification (Cooper, 1990; Gale et al., 1993; White and Reddy, 1999). Up to 77% of the variability of *in situ* rates of denitrification in a riparian wetland could be explained by the NO_3^- concentration and denitrifying enzyme activity of the soil (Schipper et al., 1993). However, carbon can also be a limiting factor if the wetland soil has a low carbon content. Significant relationships between denitrification rates and water soluble organic carbon have been shown for a number of wetland soil types (D'Angelo and Reddy, 1999). Even though NO_3^- and carbon can be limiting, the ability of denitrifying bacteria to utilize oxygen and NO_3^- as electron acceptors

gives them an ecologically competitive advantage over strictly aerobic bacteria. Many other factors, such as soil texture and structure, plants, temperature, pH, salinity, and sulfide, play a role in controlling denitrification rates in addition to organic carbon, NO_3^-, and oxygen availability. Temperature, due to climatic differences, plays a major role, with denitrification rates increasing as the temperature increases. Denitrification rates have been shown to increase 1.5 – 2.0 times with each 10°C incremental increase. Rates increase up to an optimum temperature range that occurs between 60 and 75°C, but decline rapidly above these temperatures (Knowles, 1982; Reddy and Patrick, 1984). Sulfide has been shown to inhibit nitric and nitrous oxide reductases, which are key for the completion of the denitrification process (Brunet and Garcia-Gil, 1996). Increases in salinity have been shown to lead to decreases in the rate of denitrification (Seitzinger et al., 1991; Rysgaard et al., 1999). Seitzinger, et al., (1991) showed an indirect salinity effect on denitrification caused by a reduction in nitrification rates, which was due to reduced availability of exchangeable NH_4^+. However, higher salinities are known to increase physiological stress in microbes, which could lead to a decrease in microbial diversity (de Franca et al., 2000; Yoshie et al., 2004; Grommen et al., 2005). Yoshie, et. al., (2004) have shown that salinities near that of seawater decrease the nitrite reductase gene diversity in wastewater treatment systems. Denitrification rates have been positively related to pH, with an optimum in the range of 7.0 to 8.0. Denitrification can occur up to pH 11 and down to 4. By pH 3.5, denitrification has been shown to stop in acidic peats (Knowles, 1982). At lower pHs, however, the end product of denitrification shifts from N_2 to nitrous oxide (N_2O) (Knowles, 1982; Simek and Cooper, 2002; Simek et al., 2002; Yu and Patrick, 2003). Recent evidence has suggested that the exact relationship between soil pH and the denitrification process is less clear than previously believed and that a so-called "optimum" pH range does not actually exist (Simek and Cooper, 2002; Simek et al., 2002). This is largely

due to the varying capabilities of different denitrifying microbial communities to adjust to shifts in pH, i.e., some communities are able to adapt more quickly than others.

Under some conditions there is incomplete reduction of NO_3^- to N_2, resulting in either nitric oxide (NO) or nitrous oxide (N_2O) as the main end product (DeBusk et al., 2001). Conditions influencing N_2O/N_2 production include, soil pH and redox, toxic metal stress, organic matter, NO_3^-, and O_2 content (Firestone et al., 1980; Schlesinger, 1997; Yu et al., 2001; Holtan-Hartwig et al., 2002; Yu and Patrick, 2003, 2004). Nitrous oxide plays an extremely important role in global warming as it is a strong greenhouse gas. Its radiative forcing effect is 310 times that of CO_2 and it has a lifetime in the troposphere of approximately 120 years (Kirk et al., 2004). The redox potential where denitrification becomes critical is 350 mV. For denitrification to result in significant N_2O formation, as opposed to N_2, the redox potential range is between 120 and 250 mV. The exact potential varies on the type of soil (Yu et al., 2001; Yu and Patrick, 2003, 2004). Toxic metal stress results in N_2 reduction being suppressed to a greater extent than N_2O production, so that over time, N_2O concentrations increase (Holtan-Hartwig et al., 2002). This study suggests that pollution high in metals could result in stressing of soil denitrifying microbial communities and lead to increased N_2O emissions. Holtan-Hartwig et al., (2002) point to the need of increased knowledge of the denitrifying microbial community's interactions with toxic metals. This is because microbial communities can develop a tolerance to toxic metal stress over time, but often at the loss of genetic diversity. Loss of genetic diversity can lead to reduced physiological diversity, robustness and resilience, and a greater susceptibility to environmental perturbations. The potential significance of wetland soils to global warming due to N_2O release has been extensively researched, but due to the large number of factors and complexity of interactions between them it is difficult to assess the extent to which wetland soils impact global warming.

Several different genera of bacteria have been identified that convert NO_3^- to its end product N_2, including *Pseudomonas, Alcaligenes, Flavobacterium, Paracoccus, Nitrosomonas,* and *Bacillus* (Firestone, 1982; Tiedje, 1988; Zumft, 1997; Prieme et al., 2002). The second step in denitrification reduces NO_2^- to NO by a nitrite reductase that distinguishes denitrifiers from nitrate respiring bacteria. Two different nitrite reductases are found among denitrifiers; one contains copper and is encoded by the *nirK* gene and the other contains heme *c* and heme d_1 and is encoded by the *nirS* gene (Prieme et al., 2002). No functional difference is known to exist between the two reductases. The genes *nirK* and *nirS* are often used to analyze the denitrifying microbial communities. Using these genes in combination with 16S rDNA sequence analysis, Prieme et al., (2002) explored the diversity of a denitrifying microbial community in forested upland and wetland soils. They found that the marsh soil had a more diverse denitrifying community than the upland soil and that the upland soil had a low abundance of the *nirS* gene. This is likely due to the higher organic matter content of wetlands, in addition to their fluctuating water levels, which allow for fluctuating oxygen concentrations. This study demonstrates the usefulness of studying microbial communities to further knowledge of denitrification in wetland soils.

2.2.2.8 Dissimilatory Nitrate Reduction to Ammonia

Another reaction that occurs in the anaerobic zone in wetlands is dissimilatory nitrate reduction to ammonium (DNRA). This reaction (Equation 2.9) involves the reduction of NO_3^- to NH_4^+ as opposed to the N_2 gas formed in denitrification.

$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O \qquad (2.9)$$

Whereas denitrifiers are facultative anaerobes, microorganisms that perform DNRA are thought to be obligate anaerobes (Tiedje, 1988; DeBusk et al., 2001). Conditions that favor DNRA are similar to those that favor denitrification, so it can potentially occur at the same time. However,

DNRA contributions to NO_3^- reduction are small when compared to denitrification in wetlands (DeBusk et al., 2001). Not as much is known about DNRA, but factors thought to favor it include, highly reduced soils (it consumes eight electrons as opposed to the five electrons consumed during denitrification), elevated temperatures, and high organic carbon content (DeBusk et al., 2001; Megonigal et al., 2004). Megonigal et al., (2004) presents growing evidence that DNRA may also be important in oxidized environments, so the significance of highly reduced soils is debatable. Genera of isolated bacteria that have been found to mediate DNRA include *Clostridium, Achromobacter,* and *Streptococcus* (DeBusk et al., 2001).

2.2.2.9 New Methods and Processes

Nitrogen cycling is globally important for a number of reasons. Nitrogen availability controls many aspects of global biogeochemical cycling as well as ecosystem function. Nitrogen is essential for living tissues because of its integral part of enzymes, which mediate carbon reducing or oxidizing biogeochemical reactions. It often limits the rate of primary productivity that may occur in ecosystems, which may have controlled the size and activity of the biosphere through geologic time (Schlesinger, 1997). Nitrogen also contributes to the degradation of water quality due to eutrophication. It has been estimated that the amount of plant available nitrogen would become too low to be sustainable within a week if it were not for the action of microorganisms involved in the nitrogen cycle (Maloy and Schaechter, 2006). Microbial communities are vital for transforming nitrogen, and wetlands serve as an important habitat for these microorganisms. Wetlands receive nitrogen inputs from adjacent upland areas, retain nitrogen, and, because of their location in the ecosystem, often act as nitrogen sinks. Microorganisms then transform these nitrogen inputs and serve as a driving force in the global nitrogen cycle.

Two recent studies (Baldwin et al., 2006; Cordova-Kreylos et al., 2006) discuss the use of phospholipid fatty acid (PLFA) analysis and terminal restriction fragment length polymorphism (T-RFLP) analysis to analyze how the microbial community structure is affected by potential stressors. Cordova-Kreylos et al., (2006) used these analyses to develop bioindicators of toxin-induced stress. They found that metals had a greater effect than organic pollutants on the microbial community structure from salt marsh sediments in California. Baldwin et al., (2006) looked at the effects of increased salinity on the microbial community structure in sediment from a freshwater wetland in southeastern Australia. Their findings for PLFA analysis show that the overall microbial community structure changed only at the highest NaCl loadings (100 mmol L^{-1}) and that microbial diversity decreased. The analysis of T-RFLP showed, however, that bacterial community structure had little response to increased NaCl concentrations, while archaeal community structure had significant changes with the increase of NaCl. The archaeal shift correlated with a significant decrease in the production of methane, suggesting a linkage between the microbial community structure and ecosystem function. Both of these studies indicate that an increased knowledge of the microbial community can lead to important linkages with ecosystem function in wetlands.

Much is known about nitrogen cycling in wetlands as a collective whole, but a detailed knowledge of the microorganisms affecting these processes is minimal. There are also newly discovered pathways for nitrogen reduction, anaerobic oxidation of ammonium (anammox) (Vandegraaf et al., 1995) and nitrate-dependent iron sulfide oxidation (Haaijer et al., 2006), that could potentially be important for some wetland ecosystems, but which have yet to be discovered in wetlands. Researchers are just beginning to probe the surface of understanding the diversity and composition of nitrogen-mediating wetland microorganisms. A more in-depth knowledge of the complex interactions occurring and how the microorganisms performing these processes

transpire to bring them about is needed. Understanding the wetland microbial community is also important for assessing the effects of perturbations on wetland ecosystems, such as environmental and pollutant variables (Cordova-Kreylos et al., 2006) or increasing salinity due to sea level rise (Baldwin et al., 2006). Many new techniques are enabling researchers to achieve a detailed look at microbial communities including those that are nitrogen-mediating. These techniques include 16S rRNA/DNA sequence analysis coupled with phylogeny, PLFA analysis, restriction fragment length polymorphism (RFLP) analysis, T-RFLP analysis, fluorescence *in situ* hybridization (FISH) and FISH coupled with microautoradiography (FISH/MAR), metagenomics, and stable isotope, cloning, and PCR techniques. Utilizing these techniques along with current knowledge will give a much richer view of the nitrogen-mediating microorganisms in wetlands and lead to improvements in wetland restoration efforts, constructed treatment wetlands, preservation and conservation of natural wetlands, and increased knowledge of how wetlands impact life globally.

2.3 Treatment Wetlands

2.3.1 Introduction

The natural properties wetlands possess make them ideal for wastewater treatment as most wetlands are sinks for chemicals, particularly nutrients. Treatment wetlands have been used for a variety of applications, including domestic wastewater, mine drainage, nonpoint source pollution, stormwater runoff, landfill leachate, and confined livestock operations (Gopal, 1999; Sun et al., 1999; Cardoch et al., 2000; Mitsch and Gosselink, 2000; Revitt et al., 2001; Howarth et al., 2002; Al-Omari and Fayyad, 2003; Ansola et al., 2003; Mantovi et al., 2003; Poach et al., 2003; Steinmann et al., 2003). Three types of treatment wetlands are used: natural, surface flow, and subsurface flow. Natural wetlands are existing wetlands to which wastewater is introduced. Pioneering studies in the 1970s in Florida and Michigan elevated the importance

of wetlands as “natural kidneys” in both the general public and government’s view (Ewel and Odum, 1984; Patrick, 1994; Kadlec and Knight, 1996; Verhoeven and Meuleman, 1999; Mitsch and Gosselink, 2000). As protection of wetlands increased, the need for constructed wetlands to use as an alternative to natural wetlands for wastewater treatment rose. Surface flow constructed wetlands contain low permeability soils, generally with standing water that often utilize a combination of free-floating, emergent, and submerged macrophytes. In subsurface flow constructed wetlands water flows through a matrix of gravel or soil that is very porous and generally only supports one or two emergent macrophytes. There is no standing water in these systems (Kadlec and Knight, 1996; Tanner et al., 1999; Verhoeven and Meuleman, 1999; Mitsch and Gosselink, 2000; Tanner, 2001; Weaver et al., 2003; Thullen et al., 2005; Vymazal, 2005). Only in the past 20 years, as enough data has been gathered on the safety and value of using natural wetlands, has the EPA begun to approve the use of natural wetland systems under carefully controlled conditions (Breaux and Day Jr, 1994; Day et al., 2004).

2.3.2 Marshland Upwelling System

Coastal wetland environments in Louisiana and Mississippi are typified by high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater. The majority of current onsite wastewater treatment and disposal systems technologies, such as septic systems, do not work well under these conditions. Thus, the MUS was developed as an alternative wastewater treatment system to address these special issues (Stremlau, 1994; Watson Jr. and Rusch, 2001, 2002; Richardson et al., 2004; Richardson and Rusch, 2005; Fontenot et al., 2006; Evans and Rusch, 2007b, a). The MUS is designed to utilize the prevalent natural wetlands in coastal areas. It consists of a collection/distribution tank, injection pump, programmable timer, injection well, and saturated subsurface soils (Figure 2.3). An effectively operating system is

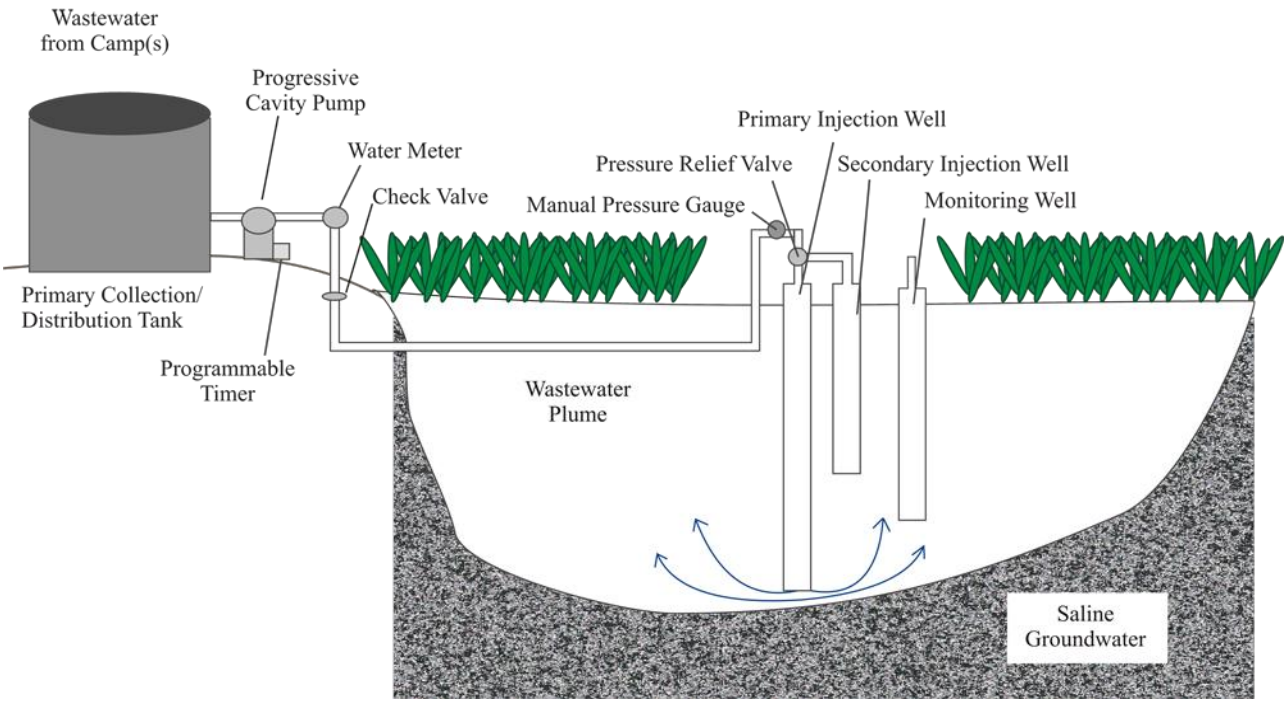


Figure 2.3 Generic schematic of the marshland upwelling system.

dependent upon the native groundwater salinity, the injection frequency and flow rate, and the natural filtering properties and microbial transformation processes of the native soil matrix.

Black and gray wastewater collected from coastal dwellings is held in a collection/distribution tank, allowing solids to settle. Settled wastewater is intermittently injected into the marsh subsurface creating active injection and resting cycles that allow pressure to dissipate during the resting cycles. The energy created by the pump radially disperses the wastewater from the injection point. As fresher wastewater is injected into a brackish or saltmarsh subsurface, the wastewater becomes a plume moved upward by buoyancy forces and laterally by natural groundwater flow. As buoyancy forces decline, the effects of lateral dispersion on plume movement become more prominent. A number of natural processes treat the wastewater as the plume moves through various oxidation-reduction zones. These processes include physical filtration, die off (of microorganisms), nutrient sorption by mineral and organic soil solids, plant uptake, and microbial decomposition and transformation. Anaerobic and anoxic

microbial processes in the subsurface coupled with aerobic processes occurring at the soil surface and rhizosphere can lead to transformations of carbon and nitrogen to gaseous forms.

Two field sites for the MUS were installed in a salt and an intermediate marsh. Various aspects of treatment have previously been studied, including the effectiveness of bacterial treatment (Watson Jr. and Rusch, 2001, 2002; Richardson and Rusch, 2005; Addo et al., 2006) along with some phosphorus and nitrogen treatment (Turriciano, 2005; Fontenot et al., 2006; Evans and Rusch, 2007b, a). A better understanding of the treatment of organic matter (as measured by CBOD) is needed. Increased knowledge of the specific processes involved in the treatment of nitrogen will help to improve treatment for future sites. The impact of salinity on treatment efficiency of the MUS is not well understood and comparisons of the effect of salinity on $CBOD_5$ and nitrogen treatment could lead to improvements in design.

CHAPTER 3: $CBOD_5$ TREATMENT USING THE MARSHLAND UPWELLING SYSTEM

3.1 Introduction

Coastal environments are home to a myriad of biological life, natural resources, and 53% of the United States' population located in 673 coastal counties (Crossett et al., 2004). Such large populations place numerous stresses on coastal environments, including surface water pollution due to wastewater discharges. Domestic wastewater increases pathogen, nutrient, and organic loads to coastal ecosystems (Ache and Wegner, 1999) resulting in reduced water and sediment quality. Thousands of hunting and fishing camps and year-round residences dot the coastal wetlands and waterways along the Gulf of Mexico. The residents who occupy these dwellings depend mainly on conventional onsite wastewater treatment and disposal systems (OTDS) or directly dispose of their wastewater into nearby wetlands or waterways. As the coastal population grows, the likelihood of increased pollution due to discharged wastewater also rises.

Once considered to have very little or no value to human society, wetlands are now recognized for the many functions and services they provide. Their ability to serve as sources, sinks, and transformers of many biological and chemical resources is invaluable (Mitsch and Gosselink, 2000; Reddy and DeLaune, 2008) and enables wetlands to remove numerous contaminants from waterbodies, which has become important as pollutants from anthropological sources continue to escalate. Wetlands have commonly been used to treat waste from a variety of sources, such as animal wastewaters (Tanner et al., 1998; Mantovi et al., 2003; Smith et al., 2006; Jou et al., 2009), shrimp processing wastewater (Cardoch et al., 2000), molasses (Sohsalam and Sirianuntapiboon, 2008), potato chip factory secondary effluent (Breaux and Day Jr, 1994), municipality secondary effluent (Breaux and Day Jr, 1994; Ansola et al., 2003),

industrial wastewater (Chen et al., 2006), and domestic wastewater (Steer et al., 2002; Al-Omari and Fayyad, 2003).

Organic matter is one of the most common pollutants targeted for reduction using treatment wetlands. The five-day carbonaceous biochemical oxygen demand ($CBOD_5$) is most often used in determining the effectiveness of most waste treatment systems for organic matter reduction, including subsurface wetlands (Kadlec and Knight, 1996; Nguyen, 2000; Vymazal, 2002; Al-Omari and Fayyad, 2003; Mantovi et al., 2003; Metcalf and Eddy, 2003; Meuleman et al., 2003; Akratos and Tsihrintzis, 2007; Jou et al., 2009). Decomposition of organic matter occurs more rapidly under aerobic conditions than the anaerobic conditions found in wetland subsurface soils (White and Reddy, 2001). However, wetland soils retard the movement of water and, subsequently, soluble organic matter flowing through the wetland subsurface has a significantly longer retention time than is generally found in open water. The longer retention time provides extended exposure of the organic matter to the microorganism consortium performing the decomposition processes in the subsurface sediments.

Coastal wetland environments in Louisiana and Mississippi are typified by high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater. The majority of current OTDS technologies, such as septic systems, do not work well under these conditions. Consequently, the marshland upwelling system (MUS) was developed as an alternative wastewater treatment system to address these special issues (Stremlau, 1994; Watson Jr. and Rusch, 2001, 2002; Richardson et al., 2004; Richardson and Rusch, 2005; Turriciano, 2005; Fontenot et al., 2006; Evans and Rusch, 2007a, b). The MUS consists of a collection/distribution tank, injection pump, programmable timer, injection well, and saturated subsurface soils (Figure 3.1). An effectively operating system is dependent upon the native groundwater salinity, the

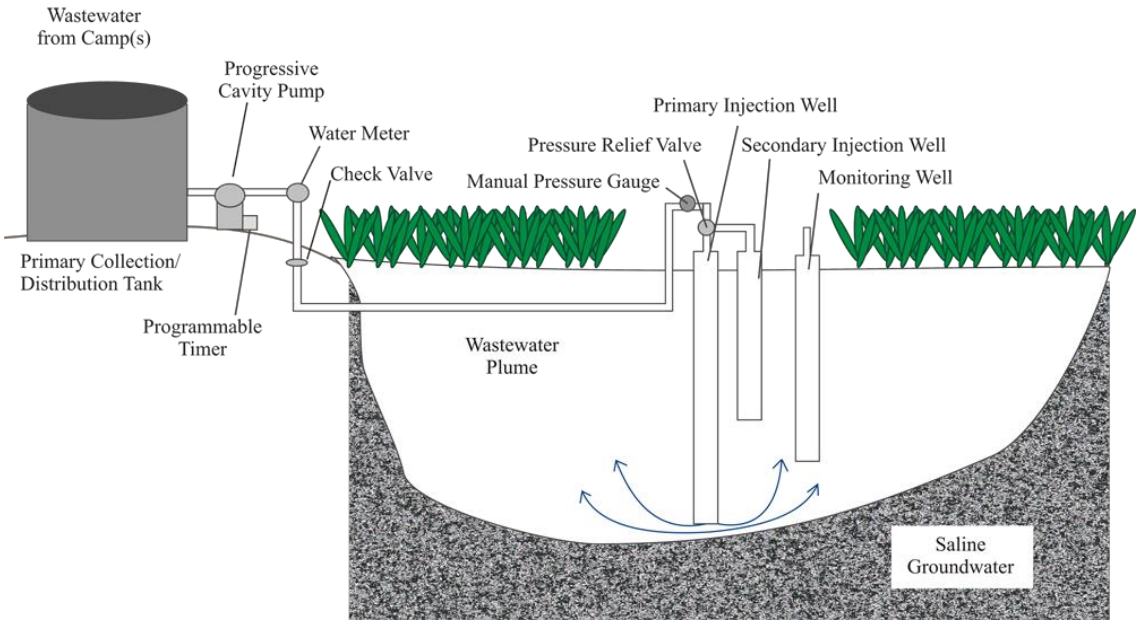


Figure 3.1 Generic schematic of the marshland upwelling system.

injection frequency and flow rate, and the natural filtering properties and microbial transformation processes of the native soil matrix.

Black and gray wastewater collected from coastal dwellings is held in a collection/distribution tank, allowing solids to settle. Settled wastewater is then intermittently injected into the marsh subsurface creating active injection and resting cycles that allow pressure to dissipate during the resting cycles. The energy created by the pump radially disperses the wastewater from the injection point. As fresher wastewater is injected into a brackish or saltmarsh subsurface, the less dense wastewater becomes a plume moving upward by buoyancy forces and laterally by natural groundwater flow. As buoyancy forces decline, the effects of lateral dispersion on plume movement become more prominent. A number of natural processes treat the wastewater as the plume moves through various oxidation-reduction zones. These processes include physical filtration, die off (of microorganisms), nutrient sorption by mineral and organic soil solids, plant uptake, and microbial decomposition and transformation.

The main objectives of this study were to: 1) evaluate the $CBOD_5$ treatment efficiency of the MUS under high (~ 30 parts per thousand, ‰) and moderate salinity (~ 10‰) groundwater

conditions, 2) determine appropriate design and operational criteria for the removal of $CBOD_5$, and 3) determine if sustained organic matter loading results in clogging.

3.2 Materials and Methods

3.2.1 Site Description

The research sites were located in close proximity to Bayou Cumbest within the Grand Bay National Estuarine Research Reserve, Moss Point, Mississippi, (abbreviated as MP) and along Bayou Segnette, Louisiana, in the Barataria Terrebonne National Estuary (abbreviated as BS). Bayou Cumbest has approximately 45 residents located directly on its banks and has been historically listed as the most impaired water body in the Mississippi List of Most Impaired Waterbodies (MDEQ, 1996). As of 2006, Bayou Cumbest remains on EPA's 303(d) list of impaired waterbodies. The MP MUS was installed within a *Juncus roemerianus* Scheele (black needlerush) saltmarsh with a mean subsurface salinity of 31‰ and serviced two privately owned camps and a public restroom. Bayou Segnette has approximately 150 residences and has suffered from poor water quality, which has led to problems for shellfish harvesting. In 1998, Bayou Segnette was listed on the EPA 303(d) list of impaired waterbodies. Bayou Segnette remained on the list as of 2006. The BS MUS site was installed in an intermediate marsh primarily vegetated by *Typha* ssp. (cattail), *Hydrocotyle ranunculoides* (water pennywort), and *Sagittaria lancifolia* (bulltongue arrowhead). The marsh had a subsurface background salinity of approximately 10‰ with a surface salinity of 1 to 2‰ and serviced a permanent, single-family residence.

3.2.2 System Description

The wastewater was a combination of gray and black water, which gravity drained into a collection/distribution tank (MP: 1,325 L; BS: 2,840 L). Solids settled out prior to injection and large solids were further prevented from entering the injection line by the installation of a

standpipe on the effluent of the tank. A low flow, high pressure, progressive cavity pump transferred the wastewater through a 1.9 cm diameter PVC line to the injection site. Wastewater injection was controlled by a programmable timer and float switch, while a water meter installed on the injection line documented the cumulative wastewater volume. Subsurface pressure was continually monitored by a pressure transducer and data logger. For the MP site, the injection well was 3.8 m deep and surrounded by 38 monitoring wells at depths of 1.5, 2.3, and 3.0 m (Figure 3.2). For BS, two injection wells at 6.1 and 4.3 m were surrounded by 25 monitoring wells at depths of 2.7, 4.0, 4.3, and 4.6 m (Figure 3.3). One well was installed away from the injection site at both study sites to monitor background conditions. Only the 4.3 m injection well was used for the BS trials. No monitoring wells were installed above 1.5 m at either site because previous studies by K. A. Rusch (personal communication) indicated surface contamination of shallow wells (0.6 to 1.0 m deep wells). Monitoring wells positioned at varying radii and vertical distances from the point of injection allowed for examination of the wastewater plume in

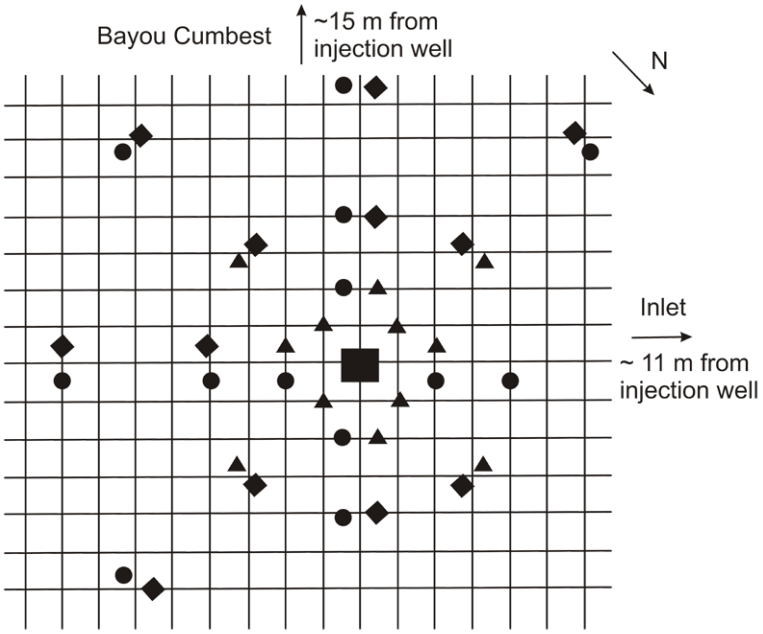


Figure 3.2 A map of Moss Point's layout, consisting of one injection well (■) and 38 monitoring wells at depths of 1.5 m (◆), 2.3 m (▲), and 3.0 m (●). Each grid square is equal to 0.61 m x 0.61 m (Fontenot, et. al., 2006). Inlet refers to wastewater holding tank.

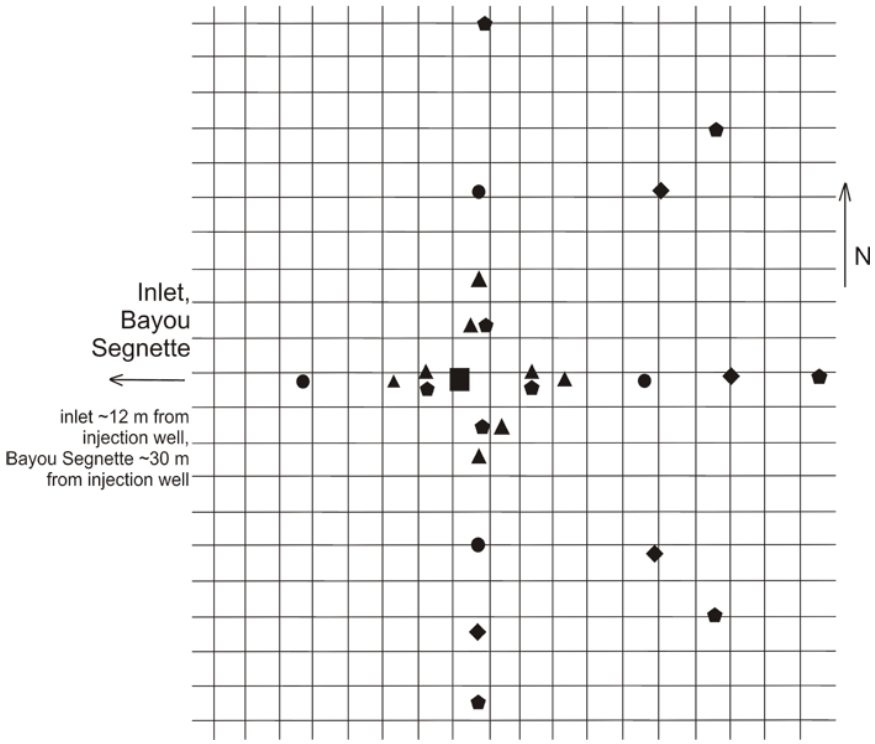


Figure 3.3 A map of Bayou Segnette's layout, consisting of two injection wells (only 4.3 m well shown (■)) and 25 monitoring wells at depths of 2.7 m (⬟), 4.0 m (◆), 4.3 m (●), and 4.6 m (▲). Each grid square is equal to 2.0 m x 2.0 m.

three dimensions. Further details on the experimental system and construction and installation procedures can be found in Watson Jr. (2000), Richardson (2002), Addo (2004), Evans (2005), and Turriciano (2005).

3.2.3 Soil Analyses

Soil corings were taken adjacent to the experimental sites and analyzed for sand, silt, and clay fractions and organic carbon content. Sand, silt, and clay fractions were measured using sieve (ASTM C117, C136) and hydrometer analyses (ASTM D422). Organic carbon content (ASTM D2974) was determined for the soil surface and at various depths (ASTM, 1995; Bardet, 1997). Organic matter was measured as loss on ignition at 435°C (Sparks, 1996).

3.2.4 Experimental Setup and Analyses

Background groundwater characteristics were evaluated before system activation to provide a baseline for system performance evaluation. System operation for MP began in June

2001 and continued through June 2002 during which time five injection flow rate and frequency serial trials were examined to determine the impact on system performance. Specific dates and corresponding trial numbers for each flow rate and frequency tested are presented in Table 3.1. All MP trials were performed at an injection frequency of 30 min every 3 h (30 min 3 h^{-1}), except for MP-5, which was performed at a frequency of 15 min h^{-1}. Flow rates were either 1.9 or 2.8 L min^{-1}, excluding MP-2 which was performed at 5.5 L min^{-1} to help determine the upper hydraulic limit of the system. Moss Point trial four was performed during the winter months with a synthetic wastewater solution that was continually dripped into the distribution tank. Synthetic wastewater was added because the camps were unoccupied during the winter months. The synthetic wastewater solution used dextrose and glutamic acid as the main carbon sources (Fontenot et al., 2006). These conditions (high loading and low temperature) combined to produce a stressed environment to help determine the functional treatment limits of the MUS. Samples were collected twice a month to monitor the $CBOD_5$ treatment capacity.

System operation for BS began in November 2002 and continued until Hurricane Katrina caused cessation of electrical power in August 2005. Three injection regimes were examined to determine the impact on system performance. Specific dates and corresponding trial numbers for each flow rate and frequency tested are presented in Table 3.1. From March 2004 to June 2005, the system was in operation, but not monitored. Bayou Segnette trials one and two were performed at an injection frequency of 15 min h^{-1}, while BS-3 was performed at a frequency of 30 min h^{-1}. Flow rates ranged from 0.95 to 2.83 L min^{-1}. Sampling was performed on a monthly basis during fall, winter, and spring months (September through May) and twice during summer months (June through August). Though there was no power (i.e., no wastewater injection into the system post-Katrina) sample collection continued (at the same frequency) until August 2006 to evaluate the ability of the subsurface environment to return to native background conditions.

Table 3.1 Injection flow rates and frequencies employed over course of the studies.

Trial number	Trial period	Duration (d)	Injection flow rate (L min^{-1})	Injection frequency (min every h)	Cumulative volume injected (L)	Theoretical hydraulic loading rate[a] (L d^{-1})
MP-1	21 June 2001 – 17 Sept. 2001	88	1.9	30 min 3 h^{-1}	13,215	456
MP-2	17 Sept. 2001 – 05 Nov. 2001	49	5.5	30 min 3 h^{-1}	7,737	1,320
MP-3	05 Nov. 2001 – 21 Jan. 2002	77	2.8	30 min 3 h^{-1}	9,236	672
MP-4	21 Jan. 2002 – 02 Apr. 2002[b]	71	2.8	30 min 3 h^{-1}	32,274	672
MP-5	02 Apr. 2002 – 03 June 2002	62	2.8	15 min h^{-1}	11,553	1,008
BS-1	10 Mar. 2003 – 03 Nov. 2003	238	0.95	15 min h^{-1}	59,977	342
BS-2	03 Nov. 2003 – 22 Mar. 2004	140	1.9	15 min h^{-1}	52,902	684
BS-3	09 June 2005 – 29 Aug. 2005	81	2.8	30 min h^{-1}	52,890	2,016

[a]Assumes an adequate volume of wastewater is present in holding tank to trigger injection.
[b]Synthetic wastewater trial.

Individual monitoring wells contained their own permanently installed, neoprene tubing that prevented cross-contamination between wells during sampling. Porewater samples were collected, stored, and transported according to standard protocols (APHA, 1998). *In situ* porewater quality parameters, which consisted of salinity, pH, and temperature, were measured for each monitoring well. Dissolved oxygen, pH, and temperature were also measured for the collection/distribution tank. Salinity was not measured in the collection/distribution tank as it was assumed to be near zero. Surface water samples were collected from the corresponding bayou during each sampling event to compare the system effluent (porewater samples pulled from monitoring wells) to ambient conditions. Salinity, dissolved oxygen, pH, and temperature were measured for the surface water. Salinity measurements were made with a conductivity meter. Redox potential was measured using platinum electrodes and a SCE reference electrode

(Patrick et al., 1996). Platinum electrodes were installed in a 1 cm PVC pipe near the monitoring wells at depths of 1.5, 2.3, and 3.0 m for MP and 2.7 and 4.6 m for BS. The tops of the access tubes were kept sealed between sampling trips. Redox measurements (E_c) were corrected to a standard hydrogen reference electrode (+245 mV) and expressed as E_h.

Unfiltered and filtered (1.2 μm GF/C glass fiber filters) samples from the collection/distribution tank were analyzed for $CBOD_5$ (method 5210 B), fecal coliforms (9222 D), total and volatile suspended solids (TSS and VSS, 2540 D and E/G), total phosphorus (TP, 4500-P B and E), orthophosphate (PO_4–P, 4500-P E), total ammonia nitrogen (TAN, 4500-NH_3 D), total Kjeldahl nitrogen (TKN, 4500-N_{org} C and 4500-NH_3 D), nitrate nitrogen (NO_3–N, 4500-NO^-_3 E), and nitrite nitrogen (NO_2–N, 4500-NO^-_2 B) (APHA, 1998). The analysis of these parameters allowed for a full characterization of the wastewater. The monitoring well samples were analyzed for $CBOD_5$, fecal coliforms, phosphorus, and nitrogen. Data for fecal coliforms, phosphorus, and nitrogen have been presented elsewhere (Richardson and Rusch, 2005; Turriciano, 2005; Addo et al., 2006; Fontenot et al., 2006; Evans and Rusch, 2007a, b).

3.2.5 Statistical Analyses

SAS® software (2009) and SigmaPlot® software (2002) were used to analyze data. All statistical tests were performed at a significance level of $\alpha = 0.05$. The influent data was used to calculate hydraulic, $CBOD_5$, and solids loading rates. Influent data from each sampling event was averaged for each trial to obtain the trial mean (± standard deviation, SD). Mean values (± SD) were calculated for *in situ* and $CBOD_5$ monitoring well data by averaging all sampling events within each trial. Data for $CBOD_5$ was also averaged by vector distance for each sampling event and then an overall trial-based mean (± standard error, SE) was calculated for each vector distance. Vector distance is defined as the distance between the bottom centroid of the injection well to the bottom centroid of a monitoring well.

The trial-based means for $CBOD_5$ were regressed against vector distance using a background corrected first-order distance removal equation (Equation 3.1) (Kadlec and Knight, 1996).

$$C = C_b + (C_o - C_b)e^{-kx} \quad (3.1)$$

where C is the effluent concentration (mg L^{-1}), C_b is the background concentration (mg L^{-1}), C_o is the influent concentration (mg L^{-1}), k is the distance-based removal constant (m^{-1}), and x is the vector distance (m). Removal constants included $CBOD_5$ treatment by dilution and microbial degradation and were determined for each trial. Predicted surface concentrations were calculated assuming the wastewater traveled directly to the surface from the end of the injection well resulting in a highly conservative estimate. Predicted travel distances to reach a National Pollutant Discharge Elimination System (NPDES) standard limit of 25 mg L^{-1} for $CBOD_5$ were also calculated. Treatment efficiencies for $CBOD_5$ were corrected for background data and calculated using the influent and monitoring wells data at 1.5 m for MP and 4.0 m for BS. The monitoring wells (vector distance of 4.6 m) at 4.0 m were used for BS because the shallowest wells (at 2.7 m) were not sampled from during the first two trials. A Weibull analysis was performed to determine the probability of exceeding a NPDES limit of 25 mg L^{-1} within the system. Individual trials were not analyzed separately as subsequent trials were dependent upon previous trials. Instead, data from the entire length of the project was included in a single Weibull analysis to provide an overall probability of exceeding the standard. For BS, only the BS-3 was used in the Weibull analysis as previous trials did not provide data for the 2.7 m depth. The value obtained from the Weibull analysis was a conservative estimate because the data used were taken from the shallowest monitoring wells at a depth of 1.5 or 2.7 m and does not reflect additional treatment in the upper sediment zones and/or rhizosphere.

3.3 Results and Discussion

3.3.1 Soil Analysis

The upper 1.2 m from the MP site consisted of dark, moderately organic soils that were consistent with Scatlake series soils, which are clayey, semifluid, predominately mineral soils that are generally poorly drained (USDA, 1984). Between 1.2 to 2.4 m, a high clay layer was identified, which could act as an aquitard. Bayou Segnette soils were consistent with the Kenner muck series, which exhibit high permeability within the organic layers and low permeability in the clay layers (USDA, 1983). For both sites, as the depth increased, the organic substrate was gradually replaced with a more compact sand/clay mixture, with the sand being predominant at increasing depth (Table 3.2). This trend was also confirmed in soil data collected from BS at a later date from the initial soil analysis (Table 3.3). Two vibra-cores were taken at BS to a depth of 6.1 m and subsequently analyzed for bulk density and organic matter content. The first 3.7 m

Table 3.2 Selected soil properties at field sites from various depths.

	Depth Interval (m)					
	Moss Point				Bayou Segnette	
Property	0-1.2[a]	1.2-2.4	2.4-3.0	3.0-3.8	0-2.7	2.7-4.6
Sand (%)	44	37	62	86	80	79
Silt (%)	44	40	23	9	16	15
Clay (%)	12	23	15	5	4	6
Fraction of organic content (f_{oc}) (%)	9.0 ± 0.5	N/A[b]	N/A[b]	0.5 ± 0.1	35.7 ± 1.2	11.6 ± 2.5
USDA classification	loam	loam	sandy loam	loamy sand	loamy sand	sandy loam

[a]Excludes plant matter.
[b]Not analyzed

Table 3.3 Soil properties from vibra-cores obtained from Bayou Segnette.

Depth Interval (m)	Bulk density (g cm^{-3})	Organic Matter Content (%)
0.0 - 2.1	0.22	41.4
2.1 - 3.7	0.57	30.9
3.7 - 4.7	1.27	1.4

had a high organic matter content and a low bulk density, but from 3.7 to 4.7 m the bulk density greatly increases and the organic matter decreases.

3.3.2 Overall System Analysis

A total of 74,015 L of wastewater was injected into the marsh subsurface for MP and 165,769 L for BS (Table 3.1). Background and experimental *in situ* and $CBOD_5$ data for both sites were monitored and compared throughout the course of the studies (Figure 3.4). Ranges of vector distances were used to compare data between the sites as vector distances were not the same for each site. The influent wastewater for both sites was characterized as medium to high strength with respect to $CBOD_5$, solids, and bacteria and high strength with respect to nutrients (Table 3.4). Unfiltered influent concentrations of $CBOD_5$ ranged from 34 to 695 mg L^{-1}, while filtered concentrations ranged from 26 to 394 mg L^{-1} for MP. For BS, concentrations ranged from 328 to 754 (unfiltered) and 128 to 557 (filtered) mg L^{-1}. The wide range in MP $CBOD_5$ values can largely be attributed to the sporadic use of the camp facilities. Moss Point trial four, which had a lower $CBOD_5$ mean than the trials immediately prior (MP-3) and after (MP-5), was performed using a mixture of glutamic acid and dextrose as a carbon source that was targeted to be 200 mg C L^{-1}. The synthetic wastewater was added because the camps were unoccupied during winter months. Influent mean ratios were calculated by averaging the ratios for individual trials to obtain one overall ratio. The mean influent solids ratios (VSS/TSS) were 0.81 and 0.82 for MP and BS, respectively, implying most of the solid load present was in the organic form. Influent $CBOD_5$ had a mean ratio (filtered/unfiltered) of 0.69 and 0.71 for MP and BS, respectively, indicating that the majority of the organic matter was present in a soluble and more readily degradable form. The presence of mostly soluble organic solids is important to the longevity of the MUS, as a more labile carbon source will be consumed faster by microorganisms and help reduce potential clogging (from organic solids) in the subsurface.

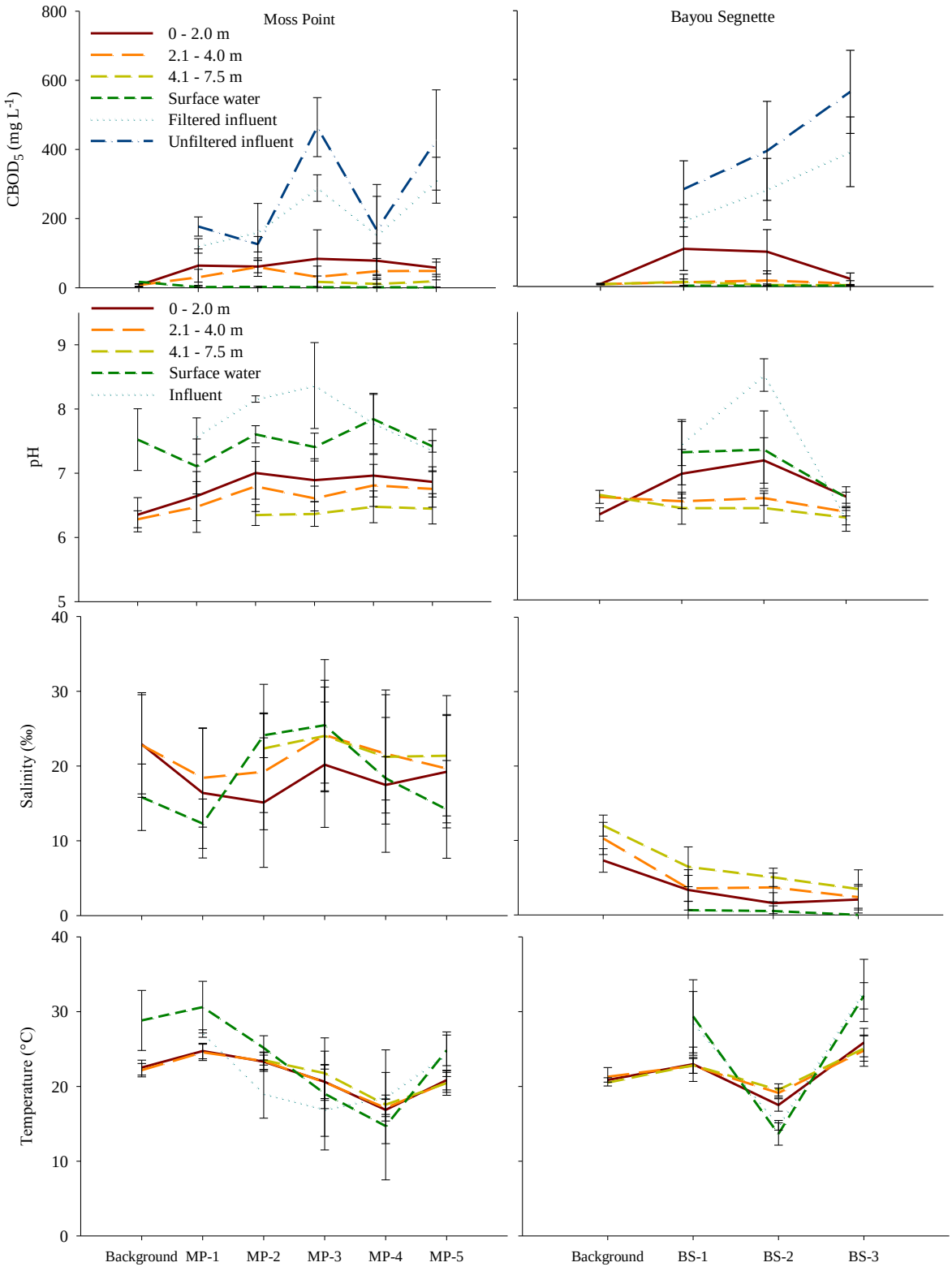


Figure 3.4 Mean *in situ* and $CBOD_5$ data (± SD) throughout the course of the study for influent, surface, and subsurface vector distance ranges. *In situ* parameters temperature, pH, and salinity are shown. Background is mean of data collected before wastewater was injected into subsurface.

Table 3.4 Influent wastewater parameters (mean ± SD).

Parameter		Results: Moss Point	Results: Bayou Segnette	Typical Wastewater[a]
Fecal coliform[b]		$3.50 \times 10^4 \pm 11$[c] (23)	$1.32 \times 10^5 \pm 5$[d] (23)	$10^4 – 10^{5,c}$ $10^3 – 10^{8,d}$
TAN (mg L^{-1})	Filtered	115 ± 59 (21)	97 ± 30 (26)	12 – 50
	Unfiltered	141 ± 53 (14)	99 ± 32 (26)	
TKN (mg L^{-1})	Filtered	124 ± 71 (21)	108 ± 31 (25)	8 – 35
	Unfiltered	155 ± 72 (16)	121 ± 38 (25)	
NO_2-N (mg L^{-1})		0.13 ± 0.16 (15)	0.10 ± 0.06 (25)	0
NO_3-N (mg L^{-1})		BDL[e] (13)	BDL (21)	0
TP (mg L^{-1})	Filtered	15.6 ± 10.0 (21)	12.5 ± 3.7 (23)	4 – 15
	Unfiltered	17.4 ± 10.1 (19)	15.1 ± 5.3 (19)	
PO_4-P (mg L^{-1})	Filtered	11.6 ± 6.9 (17)	11.3 ± 3.3 (25)	1 – 5
	Unfiltered	11.7 ± 7.0 (15)	not available	
$CBOD_5$ (mg L^{-1})	Filtered	217 ± 104 (21)	257 ± 108 (26)	110 – 400
	Unfiltered	282 ± 173 (24)	366 ± 151 (25)	
TSS (mg L^{-1})		147 ± 93 (23)	238 ± 226 (19)	100 – 350
VSS (mg L^{-1})		111 ± 61 (22)	183 ± 177 (19)	80 – 275
pH		7.80 ± 0.52 (21)	7.27 ± 0.76 (24)	--
Dissolved oxygen (mg L^{-1})		1.25 ± 2.20 (18)	1.49 ± 0.81 (21)	--
Temperature (°C)		22.0 ± 2.8 (20)	27.4 ± 6.7 (25)	--

Table is modified from Fontenot, et. al. (2006). ( ) = n.
[a]Metcalf and Eddy (2003).
[b]Geometric mean ± geometric standard deviation.
[c]colonies 100 ml^{-1}.
[d]MPN 100 ml^{-1}.
[e]Below detection limit; 0.05 mg L^{-1}.

Salinity decreased with vector distance at both sites (Figure 3.4). The decreasing trend with vector distance was due to the incoming wastewater, which was assumed to have a salinity near 0‰. There did not appear to be any long-term trends in the data at MP; however, BS showed a continued decreasing trend in salinity over time (Fontenot et al., 2006; Evans and Rusch, 2007a). Background salinity averaged 31 and 9‰ for MP and BS, respectively, before sampling occurred and was significantly higher than all experimental trials, indicating the input of wastewater decreased salinity in the plume area. Fluctuations and the lack of a continued

salinity decrease (at MP) were most likely due to some influence of the surrounding groundwater as well as local tidal influx, which was suggested by the close tracking of the groundwater with the surface water. The trends observed from the other *in situ* parameters measured suggested salinity was the best indicator of plume movement, in agreement with previous studies (Richardson et al., 2004; Evans and Rusch, 2007b).

3.3.3 $CBOD_5$ Removal as a Function of Depth and Vector Distance

Influent samples measured for $CBOD_5$ totaled 47 (51) [MP (BS)], with 382 (325) samples collected from the monitoring wells. Over the course of the projects, 253 (152) monitoring well samples, or 66% (47%), had $CBOD_5$ levels over the NPDES limit of 25 mg L^{-1}. The inner circle of monitoring wells had 82%, n=124, (22%, n = 17) of samples at or above the limit, while monitoring wells at the outermost wells, showed, on average, only 70%, n = 68, (5%, n = 5) had concentrations at or above the limit. The drop from 82 to 70% (22 to 5%) demonstrated that as the wastewater moves laterally up and away from the injection well, $CBOD_5$ levels were reduced. A Weibull analysis showed the probability of exceeding the 25 mg L^{-1} standard at the 1.5 m wells was 38% for MP and < 0.01% at the 2.7 m wells for BS. It should be noted that these wells were 1.5 or 2.7 m below the surface and the probabilities were, therefore, conservative estimates. Further treatment would occur in the remaining 1.5 or 2.7 m. In addition, the rhizosphere would be expected to increase treatment as it harbors larger microbial consortiums near plant roots. Thus, the probability of exceeding the standard would be expected to decrease as the wastewater moved closer to the surface.

Removal efficiencies for $CBOD_5$ are presented in Table 3.5. Recall treatment efficiency was calculated using data from the 1.5 or 4.0 m monitoring wells, so that 1.5 or 4.0 m of soil remained for wastewater to travel through, for MP and BS, respectively, plus any additional removal by plants at the surface. The lower removal efficiency of trial one at both sites can be

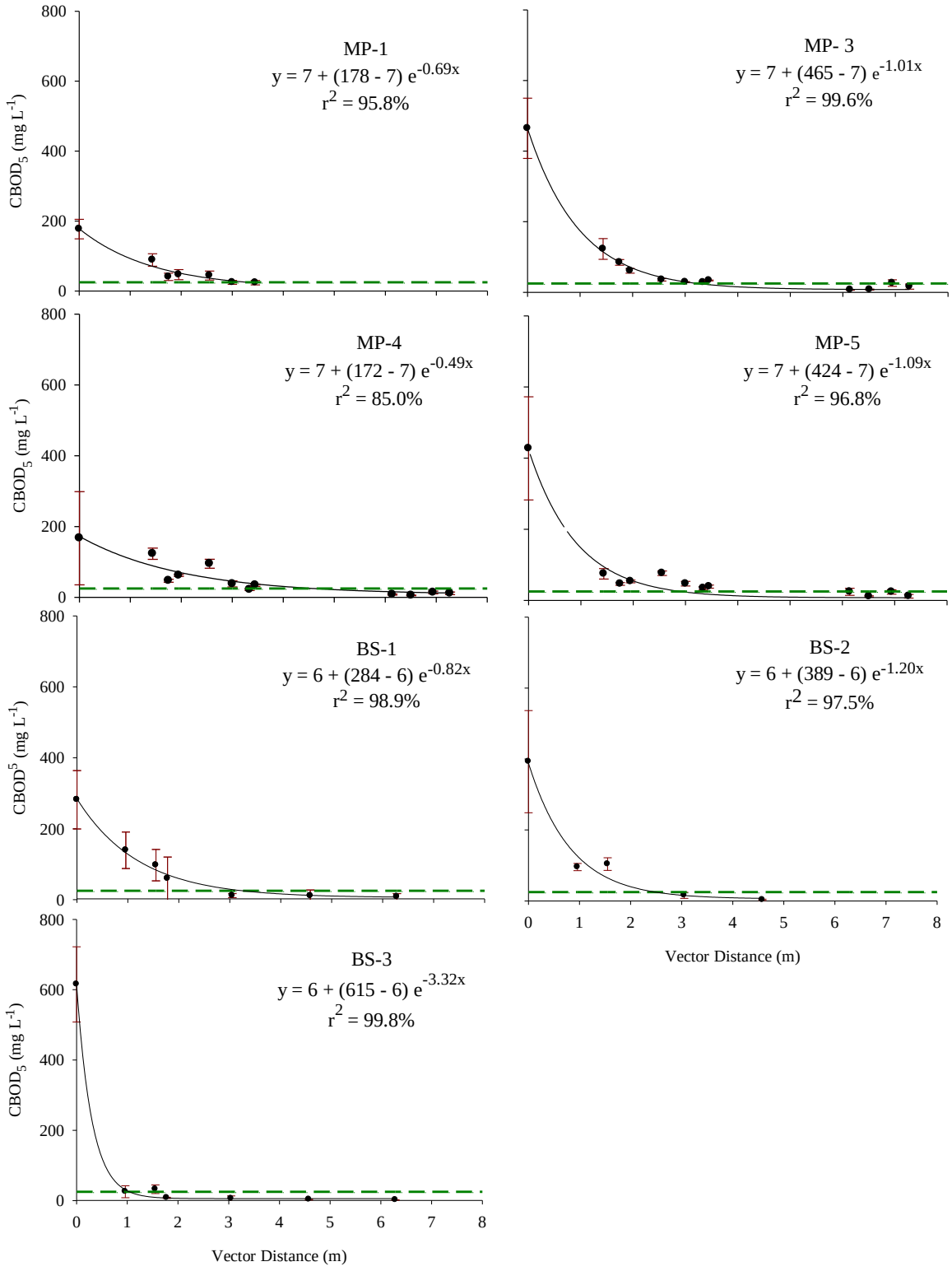
Table 3.5 Predicted $CBOD_5$ removal as a function of vector distance from injection point. Removal constants were used to calculate predicted surface concentrations, which are conservative values calculated assuming the wastewater moves directly from the point of injection to the surface, and predicted travel distances, which provide an estimate of how far the wastewater must travel to reach the $CBOD_5$ NPDES limit of 25 mg L^{-1}. Global removal constants were found from fitting a first-order equation to all $CBOD_5$ data at each site as opposed to a mathematical average.

Trial	Injection depth (m)	Removal efficiencies (%)	First-order removal constant (m^{-1})	Predicted surface concentration (mg L^{-1})	Predicted travel distance (m)
MP-1	3.8	90	0.69	19.8	3.3
MP-3		96	1.01	17.3	3.2
MP-4		93	0.49	33.0	4.6
MP-5		96	1.09	14.1	2.9
Global MP		**95**	**0.80**	**20.4**	**3.4**
BS-1	4.3	97	0.89	11.7	3.0
BS-2		> 99	1.21	7.8	2.5
BS-3		> 99	3.32	5.7	1.0
Global BS		**99**	**1.30**	**7.0**	**2.3**

accounted for by the limited acclimation, at that point, of the subsurface to system conditions, but even these trials are within the range of BOD removal efficiencies from other studies of constructed subsurface wetlands (Vymazal, 2002; Al-Omari and Fayyad, 2003; Mantovi et al., 2003; Meuleman et al., 2003; Sohsalam and Sirianuntapiboon, 2008; Jou et al., 2009). A vertical flow constructed wetland treating domestic and recreational wastewater (Meuleman et al., 2003) and horizontal subsurface flow constructed wetlands treating dairy parlor, domestic and municipal wastewater (Vymazal, 2002; Mantovi et al., 2003) achieved removal efficiencies from 84 to 96%. Surface flow constructed wetlands treating molasses and animal-contaminated creek water had removal efficiencies of 66 to 89% (Sohsalam and Sirianuntapiboon, 2008; Jou et al., 2009). In contrast, a subsurface flow constructed wetland study (Al-Omari and Fayyad, 2003) had much lower removal efficiencies treating domestic wastewater, ranging from 33 to 65%. A surface flow wetland treating dairy wastewater achieved removal efficiencies of 99% (Smith et

al., 2006), while a free water surface wetland treating industrial wastewater achieved removal efficiencies ranging from 51 to 89% (Chen et al., 2006). In comparison to conventional septic systems the MUS performed better, as septic system BOD removal efficiencies ranged from 30 to 50% for wastewater from single-family homes, small communities, and cluster systems (USEPA, 1999, 2002). However, the environment in which the MUS and septic systems work is not similar and this should be taken into account when comparing removal efficiencies.

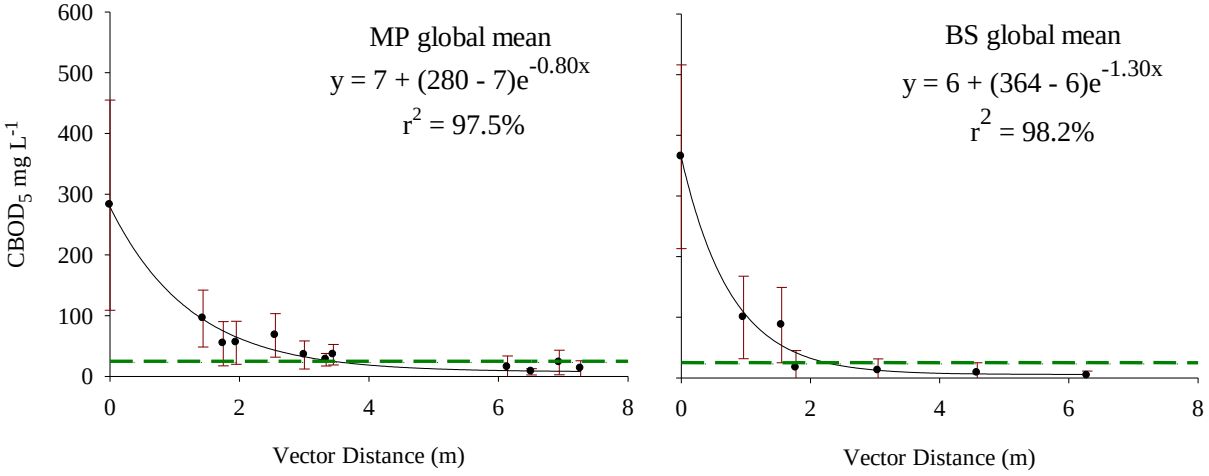
Removal of $CBOD_5$ was also analyzed by vector distance using a background-corrected first-order removal equation. Removal constants, predicted surface concentrations, and travel distances were calculated (Table 3.5). Selected removal curves (Figure 3.5) show the $CBOD_5$ concentration by vector distance. The removal constants at both sites increased with time, excluding the MP synthetic wastewater trial (MP-4), indicating that treatment effectiveness also increased with time. At MP, the higher removal constants seen for MP-3 and 5 were largely due to the higher influent CBOD concentrations these trials had in comparison to MP-1 and 4. Trial three at BS had the highest influent CBOD concentration, which may explain the higher removal constant seen for BS-3 versus BS-1 and 2. However, with a lower influent CBOD concentration, the subsurface concentration would also be expected to be lower if treatment were similar. A possible explanation for trial one at both sites having lower removal constants could be a lag time required by the microbial community to acclimate to the new conditions. During MP-4, synthetic wastewater was added continually (because of low camp usage) and provided a more easily degradable substrate. A lower influent CBOD concentration and more labile carbon source would be expected to produce a greater drop in CBOD than was observed. The continual input of wastewater likely accounted for this discrepancy, as the less efficient anaerobic microbial population may not have been able to degrade the wastewater at a high enough rate to keep up with the increased CBOD loading rate, especially under low temperature conditions.



(a)

Figure 3.5 Carbonaceous BOD_5 removal with respect to vector distance from injection well. (a) Data from individual trials. (b) Data from global means. The given equation is the predicted equation based on collected data. Each data point represents mean $CBOD_5$ concentration at given vector distance. Error bars represent standard error for each data point. The dashed line shows the $CBOD_5$ NPDES limit of 25 mg L^{-1}.

(Figure 3.5 con'd)



(b)

Therefore, it seems likely that as the microbial population acclimated to the incoming wastewater, treatment would improve, and could potentially increase as indicated by removal constants from MP-1, 3, and 5 and BS-1 through 3.

Microbial growth and activity are impacted by a number of factors, including salinity, temperature, and substrate type. The differences in organic matter degradation between MP and BS could also be affected by differences in salinity, temperature, and soil matrix. Increases in salinity have been shown to lead to decreases in the microbial biomass under various soil conditions (Sardinha et al., 2003; Wichern et al., 2006; Yuan et al., 2007a; Wu et al., 2008). Higher salinities lead to increases in the physiological stress of microbes, which could lead to decreases in microbial diversity and, subsequently, microbial activity (de Franca et al., 2000; Yoshie et al., 2004; Grommen et al., 2005). Though there was no significant difference seen between the removal efficiencies and constants for each site, there was a trend for BS to have higher removal efficiencies and constants than MP. A higher subsurface salinity at MP could be the cause of the differences seen in removal efficiencies and constants between the two sites. Lower temperatures can negatively impact the growth and activity of the microbial community (Heal et al., 1981; D'Angelo and Reddy, 1999). The trials that occurred during winter months

(MP-4 and BS-2), also had lower removal efficiencies and decay constants than those trials (MP-4, MP-5, and BS-3) that occurred during warmer months for each site.

While microbial degradation accounted for some CBOD removal, the removal constant also reflected dilution effects on organic matter reduction. A carbon mass balance analysis with a first-order decay component can be used to estimate the effect of dilution on the removal constant. Carbon transport into the treatment area would come from the injected wastewater and the carbon contained in the native groundwater. The wastewater and native groundwater organic content are easily measurable and known. While the injection flow rate is a known, the native groundwater velocity may or may not be known, but can be estimated. Since the MUS does not have rigid boundaries, an estimation of the porous area/volume must be made. The only unknown in the analysis would be the decay coefficient, which could be determined under steady state conditions. Once calculated, a comparison with the estimated removal constants might reveal how much of the removal was due to decay and how much was due to potential dilution.

As an example, consider the BS site. Groundwater velocities in the general area of the treatment site were measured to be 1.6, 2.3, and 9.6 cm d^{-1} (Inniss, 2002). The mean influent and native groundwater $CBOD_5$ levels were 366 and 5.65 mg L^{-1}. The mean injection flow was 0.43 m^3 d^{-1} and the treatment area (porous) was estimated to be 320 m^3. Solving the mass balance under steady state conditions yields decay constant values of 0.07, 0.10, and 0.17 m^{-1} for BS-1, 2, and 3, respectively. Comparing these values to the removal constants in Table 3.5, microbial degradation appears to have a minimal impact on $CBOD_5$ treatment. However, the exact volume of the treatment area is unknown. It is unlikely the treatment area was a perfect cube of the dimensions used in the estimation. If half of the cube volume is used, the decay constants increase to 0.14, 0.20, and 0.35 m^{-1} for BS-1, 2, and 3. This analysis shows that both dilution and degradation are contributing to the reduction of organic matter within the MUS.

Removal constants were used to calculate predicted surface concentrations and travel distances. Predicted surface concentrations were calculated assuming the wastewater moves directly from the point of injection to the surface (3.8 or 4.3 m, MP and BS, respectively). An outflow concentration cannot be provided for the MUS as monitoring wells above 1.5 m could not be installed. However, the predicted surface concentrations can provide a conservative estimate of the outflow concentration. Predicted travel distances provided an estimate of how far the wastewater must travel to reach the $CBOD_5$ NPDES limit of 25 mg L^{-1}. Predicted surface concentrations and travel distances both decreased with each subsequent trial, which also demonstrated improved treatment as time progressed. The predicted surface concentrations for all trials (Table 3.5), except MP-4, were below the NPDES $CBOD_5$ limit of 25 mg L^{-1} and travel distances were less than the 3.8 or 4.3 m depth of the injection well. The high strength of the synthetic wastewater explains why MP-4 performed the worst in terms of removal constants; however, it did provide a significant reduction of the $CBOD_5$. Predicted surface concentration and travel distances are only estimates of actual treatment capabilities and, while the predicted surface concentration may be 33 mg L^{-1}, this number was still close to the 25 mg L^{-1} limit. It should be noted that the synthetic wastewater trial (MP-4) occurred under highly improbable conditions, i.e., high loading conditions with low temperatures. The predicted surface concentration of 33 mg L^{-1} and the actual value of 19 mg L^{-1} (for MP-4) from a depth of 1.5 m further reiterates the conservative nature of these estimates. The predicted travel distances were also based on the assumption that wastewater traveled directly to the surface from the bottom of the injection well. Unless the system has been stressed and channelization has occurred, wastewater would travel laterally away and upwards from the injection well and not directly up to the surface. Also, the actual path, assuming there has been no channelization, would be a much more tortuous and longer one as the wastewater moved in and around the soil particles.

Tortuosity is difficult to measure and highly dependent upon the type of sediment present (Boudreau and Meysman, 2006). Within the MUS, sand, silt, and clay were all present and in varying amounts depending on depth. Actual implementation of the MUS as a wastewater treatment system should take path length into consideration when determining the injection depth, as sands, silts, and clays will have different path or travel lengths based on tortuosity. A sandier soil would have a lower tortuosity and, thus, a shorter path length and require a deeper injection depth than a more clayey soil to ensure adequate $CBOD_5$ treatment.

No trend was observed relating injection frequency and flow rate to $CBOD_5$ treatment. Trial one at MP had the lowest flow rate (at MP) and would be expected to have a higher removal efficiency and removal constant than MP-3 and 5, which had flow rates double that of MP-1. Likewise, MP-5 would be expected to have a lower removal efficiency and removal constant as its injection frequency was higher than that of trial one and three. However, MP-1 had the lowest removal efficiency (90%) and removal constant (0.69 m^{-1}), while MP-3 (96% and 1.01 m^{-1}, respectively) and MP-5 (96% and 1.09 m^{-1}, respectively) were very similar. At BS, a similar trend is noticed. The lowest flow rate trial (BS-1) would be expected to have the highest removal efficiency and removal constant, while the highest flow rate trial (BS-3) would be expected to have the lowest as it has the highest injection frequency. But the exact opposite occurred, with BS-3 having the highest removal efficiency (> 99%) and removal constant (3.32 m^{-1}) and BS-1 having the lowest (97% and 0.89 m^{-1}, respectively). This suggests an acclimation period of the microbial population was initially more important for the treatment of organic matter than a particular injection regime and that treatment improved over time.

3.3.4 Implications for System Design and Operation

Hydraulic, $CBOD_5$, TSS, and VSS loading rates were calculated for each trial (Table 3.6). Mean hydraulic loading rates (HLR), which varied from 144 to 756 L d^{-1}, were less than

Table 3.6 Mean daily hydraulic, $CBOD_5$ (unfiltered), and solids loading rates for influent wastewater are presented for each trial. Global means are the mean (± SE) for the trials presented.

Trial	Hydraulic loading rate ($L\ d^{-1}$)	$CBOD_5$ loading rate ($g\ d^{-1}$)	TSS loading rate ($g\ d^{-1}$)	VSS loading rate ($g\ d^{-1}$)
MP-1	150	18	12	9
MP-3	120	35	23	16
MP-4	454	48	122	101
MP-5	186	58	24	20
Global MP	**228 ± 154 (4)**	**40 ± 17 (4)**	**45 ± 51 (4)**	**37 ± 43 (4)**
BS-1	252	67	45	32
BS-2	378	148	no data	
BS-3	653	387	275	231
Global BS	**428 ± 205 (3)**	**201 ± 166 (3)**	**160 ± 163 (2)**	**132 ± 141 (2)**

the theoretical HLR for MP and BS (Table 3.1). Theoretical HLRs were calculated assuming there was a continuously adequate amount of wastewater. Actual HLRs ranged from 18 to 68% of theoretical HLRs for MP, indicating the system was capable of meeting the daily wastewater flow requirements of the camps at MP. At BS, HLRs ranged from 32 to 74% of the theoretical HLRs, which also indicated BS was capable of meeting the daily requirements of the camp. Loading rates were calculated as $g\ d^{-1}$ instead of $g\ m^{-3}\ d^{-1}$ because there are no boundaries to the MUS and the extent of the treatment area is very difficult to measure. The highest MP HLR occurred during MP-4, which used synthetic wastewater and a continual input to achieve maximum loading rates and was reflected in the high $CBOD_5$, TSS, and VSS loading rates (CLR, TLR, and VLR, respectively) for this trial. Excluding MP-4, mean CLRs ranged from 26 to 58 $g\ d^{-1}$, mean TLRs ranged from 18 to 27 $g\ d^{-1}$ and mean VLRs from 13 to 20 $g\ d^{-1}$. Loading rates for BS ranged from 156 to 447, 105 to 318, and 74 to 267 $g\ d^{-1}$ for CLR, TLR, and VLR respectively. There were no significant differences found between global means.

Although these were serial studies that were not independent of each other, some general trends were observed. Moss Point trial four occurred under conditions of high hydraulic and carbon loading (Table 3.6) and low temperatures (Figure 3.4) and resulted in a lower removal efficiency (93%) than the trials before (MP-3) and after (MP-5), both of which had higher removal efficiencies (96%) and lower loading rates. Lower temperatures combined with high loading rates may have played a role in the decreased removal efficiency of the synthetic wastewater trial and is an important consideration for any future use of the MUS. The data at BS, however, did not follow the same trend. The trial with the lowest loading rates (BS-1) had the lowest removal efficiency (97%), while both subsequent trials (BS-2 and 3) had higher loading rates and higher removal efficiencies (> 99%). The trend at BS further supports microbial degradation as part of the removal mechanism for CBOD, as the loading rates for these trials are high, thus reducing the effect of dilution, and removal efficiencies are near 100%.

Hurricane Katrina provided the opportunity to observe subsurface conditions after system shutdown and to determine the time necessary to return to background conditions. Levels of $CBOD_5$ dropped consistently to below the 25 mg L^{-1} limit; however, a year after Katrina they had not returned to background conditions within the first 2 m vector distance (Figure 3.6). These levels indicated that the subsurface reached a new steady state, as levels did not continue to drop, but rather stayed around 15 mg L^{-1}. The new steady state could be due to slow hydrolysis of solids releasing soluble carbon. It is important to note that at the conclusion of this study, levels were higher than previous background conditions, but they were still below NPDES limits.

The long-term success of the MUS relies not only on the system's ability to treat wastewater constituents, but on longevity of operation. The solids laden wastewater may eventually lead to chronic clogging of the area surrounding the injection well. This has not been

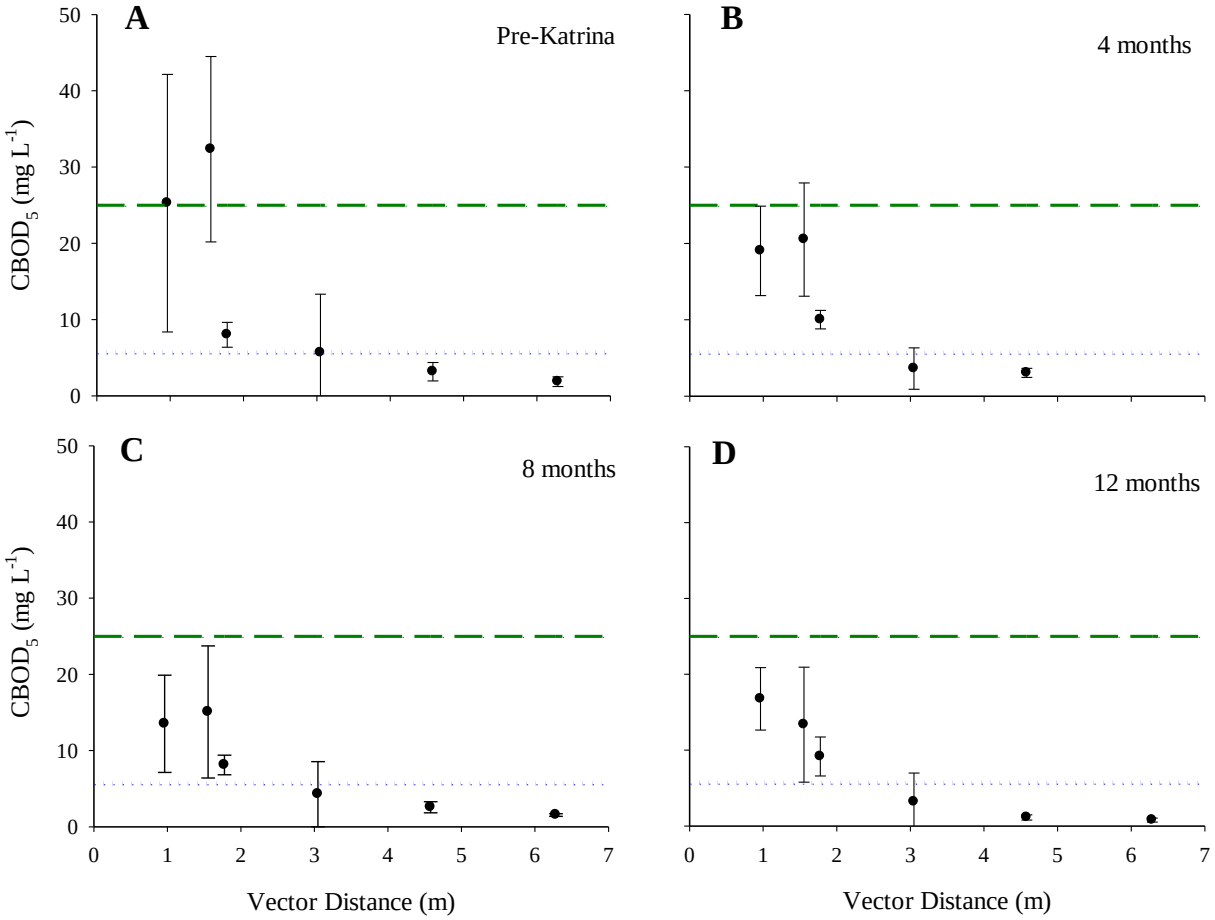


Figure 3.6 Carbonaceous BOD_5 by time for pre and post-Katrina with respect to vector distance from injection well. Graph A shows pre-Katrina data, B shows 4 months post-Katrina, C shows 8 months post-Katrina, and D shows 12 months post-Katrina. Each data point represents mean $CBOD_5$ concentration at given vector distance. Error bars represent standard error for each data point. The dashed line shows the $CBOD_5$ NPDES limit of 25 mg L^{-1}. The dotted line shows the initial background $CBOD_5$ of 5.65 mg L^{-1}.

observed by the investigators during the course of these studies. Estimating the longevity of the system is complex and must include an analysis of the wastewater strength, soil characteristics, natural ground water flow, and salinity. In addition, the actual mode of wastewater injection may have an impact on the potential for clogging. Currently, the MUS utilizes one active well (point injection) with one back-up well and while this strategy has worked to date, increasing the surface area for injection via horizontal injection lines versus vertical point injection should be investigated. There was some indication for an increase in subsurface organic matter with continued wastewater input (Figure 3.6). If this is the case long-term, continued wastewater

input could lead to clogging issues and eventually result in channelization. Aeration of the wastewater while still in the collection/distribution tank could lead to decreases in the amount of organic matter injected into the subsurface and, thus, increase the longevity of the system. Though no impacts of injection flow rate and frequency on $CBOD_5$ treatment were observed, hydraulic channelization directly to the surface from the bottom of the injection well was observed during MP-2, when the upper hydraulic limits were tested. Thus, the injection flow rate should be considered during operation of the system. However, the MUS still provided significant treatment of CBOD and worked well for the conditions present in coastal environments.

3.4 Conclusion

The results of these studies indicate that the MUS can effectively treat the organic matter component of wastewater. Treatment below the NPDES standard $CBOD_5$ limit of 25 mg L^{-1} is achievable for coastal dwelling wastewater within a vector distance of 7 m (MP) or 3 m (BS) from the injection site. Removal efficiencies ranged from 90 to > 99%, reducing the $CBOD_5$ from a mean of 296 to 23 mg L^{-1} for MP and 365 to 8 for mg L^{-1} BS. Both salt and intermediate marshes provide effective treatment of organic matter. Though there were no significant differences found between sites, global data (Tables 3.5 and 3.6) indicates there may be a trend for a lower salinity marsh to treat organic matter more effectively. However, due to differences in influent wastewater characteristics and subsurface environments, more data would be needed to make any definitive conclusions on the impact of salinity on organic matter treatment. Injection frequency and flow rate appear to have no affect on $CBOD_5$ treatment. Success of the MUS for $CBOD_5$ treatment appears to be partially dependent upon the microbial consortium present, in addition to the effects of dilution, as can be seen from the improvement in treatment as the MUS acclimates. Therefore, the length of the acclimation period should be considered

during system design. No clogging or hydraulic failure was observed during the course of either study, which was shown by the lack of increase in injection pressure throughout the study (data not shown). Although our results support the use of the MUS technology for treatment of wastewater organic carbon, several other parameters, such as bacteria, phosphorus, and nitrogen, should be considered for a more effective system design. Future studies will need to address these concerns before this type of system can be implemented as an ecologically responsible method of waste management. However, the MUS shows good potential for treatment of wastewater in coastal wetland environments.

CHAPTER 4: A LABORATORY MACROCOSM STUDY OF NITROGEN TREATMENT WITHIN THE MARSHLAND UPWELLING SYSTEM

4.1 Introduction

Nitrogen is an essential component in biological systems and is found in amino acids, DNA, enzymes, and a host of other materials essential for life. However, certain forms of nitrogen, such as ammonium (NH_4^+) and nitrite (NO_2^-), can be toxic to some aquatic species (Camargo and Alonso, 2006; Paerl, 2006; Burkholder et al., 2007). Nitrogen, in its various forms, can undergo several different microbial transformations, potentially leading to a reduction of nitrate (NO_3^-) to dinitrogen gas (N_2) or nitrous oxide (N_2O), depending on environmental conditions. Conversion to N_2O would be problematic as N_2O is a potent greenhouse gas and transferring significant amounts to the atmosphere would be undesirable. Nitrogen discharge into waterbodies is of concern when concentrations are excessive, which can lead to eutrophication. However, the level of particular nutrients required to cause eutrophication varies (Camargo and Alonso, 2006; Burkholder et al., 2007). Some ecosystems are more nutrient limited than others and, thus, concentrations necessary to bring about eutrophication differ. Because of the various forms of nitrogen and their functions, nitrogen cycling is vitally important to global biogeochemical cycling.

In wetlands, the juxtaposition of aerobic and anaerobic zones allows for a unique coupling of several processes not often found to occur in close range elsewhere in nature. Wetlands were once considered to have very little or no value to human society, but are now recognized for the many functions and services they provide. They serve as habitat and protection for a number of fish and wildlife species; as sources, sinks, and transformers of many biological and chemical resources; as buffers from storm surges (as wetlands can mitigate

flooding); as a mechanism to recharge and discharge groundwater; and as a way to stabilize sediment and prevent erosion (Patrick, 1994; Woodward and Wui, 2001).

The natural properties wetlands possess make them ideal for wastewater treatment as most wetlands are sinks for chemicals, particularly nutrients. Treatment wetlands have been used for a variety of applications, including domestic wastewater, mine drainage, nonpoint source pollution, stormwater runoff, landfill leachate, and confined livestock operations (Gopal, 1999; Sun et al., 1999; Cardoch et al., 2000; Revitt et al., 2001; Howarth et al., 2002; Al-Omari and Fayyad, 2003; Ansola et al., 2003; Mantovi et al., 2003; Poach et al., 2003; Steinmann et al., 2003). Pioneering studies in the 1970s in Florida and Michigan elevated the importance of wetlands as "natural kidneys" in both the general public and government's view (Ewel and Odum, 1984; Patrick, 1994; Verhoeven and Meuleman, 1999).

The marshland upwelling system (MUS) was developed as an alternative wastewater treatment system to treat domestic wastewaters in coastal wetland environments (Stremlau, 1994; Watson Jr. and Rusch, 2001, 2002; Richardson et al., 2004; Richardson and Rusch, 2005; Fontenot et al., 2006; Evans and Rusch, 2007a, b). Coastal wetland environments in Louisiana are typified by high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater. The MUS is designed to utilize the prevalent natural wetlands in coastal areas. The system consists of a collection/distribution tank, injection pump, programmable timer, injection well, and saturated subsurface soils (Figure 4.1). An effectively operating system is dependent upon the native groundwater salinity, the injection frequency and flow rate, and the natural filtering properties and microbial transformation processes of the native soil matrix.

Black and gray wastewater collected from coastal dwellings is held in a collection/distribution tank, allowing solids to settle. Settled wastewater is intermittently injected into the marsh subsurface creating active injection and resting cycles that allow pressure

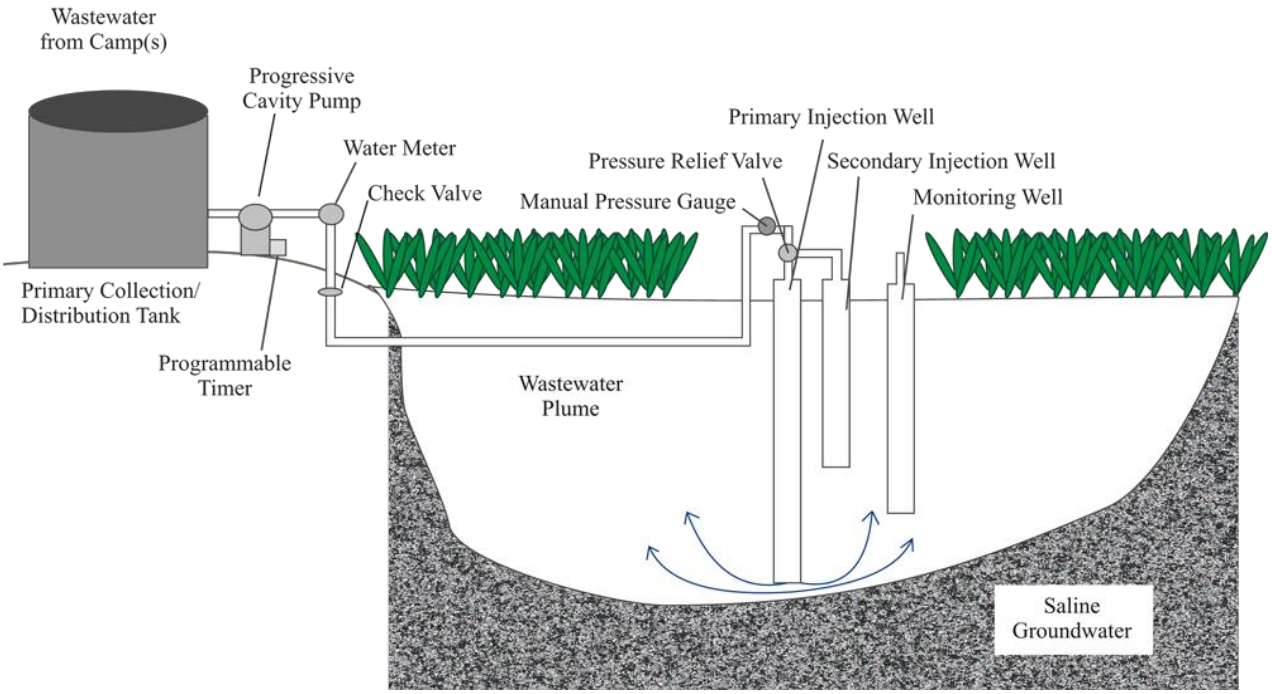


Figure 4.1 Generic schematic of the marshland upwelling system.

to dissipate during the resting cycles. The energy created by the pump radially disperses the wastewater from the injection point. As fresher wastewater is injected into a brackish or saltmarsh subsurface, the less dense wastewater becomes a plume moving upward by buoyancy forces and laterally by natural groundwater flow. As buoyancy forces decline, the effects of lateral dispersion on plume movement become more prominent. A number of natural processes treat the wastewater as the plume moves through various oxidation-reduction zones. These processes include physical filtration, die off (of microorganisms), nutrient sorption by mineral and organic soil solids, plant uptake, and microbial decomposition and transformation. Anaerobic and anoxic microbial processes in the subsurface coupled with aerobic processes occurring at the soil surface and rhizosphere can lead to transformations of carbon and nitrogen to gaseous forms.

One of the goals of the MUS is the removal of nutrients associated with the wastewater (Fontenot et al., 2006; Evans and Rusch, 2007a, b). In order to look at nitrogen removal from the system in a more controlled environment, a laboratory experiment was designed to simulate

MUS conditions in the field. The main objectives of the laboratory study were to: 1) determine the fate of nitrogen within the MUS, 2) determine the impact of plants on nitrogen treatment, and 3) evaluate nitrogen treatment efficiency of the MUS under high (~ 20 parts per thousand, ‰) and low (~ 2‰) salinity conditions.

4.2 Materials and Methods

4.2.1 Experimental Setup

Nitrogen transformations within the MUS were investigated using a laboratory column experiment because field conditions are essentially unbounded, thus preventing a quantitative and detailed process investigation. The removal of nitrogen was evaluated under varying conditions chosen to simulate field conditions as closely as possible. Three experimental treatments (salinity, nitrogen, and plants), with two levels each were chosen to evaluate the efficiency of nitrogen removal (Figure 4.2). The nitrogen levels were chosen as representative of levels seen at field sites (Turriciano, 2005; Fontenot et al., 2006). To determine the amount of NO_3^- to use, a small laboratory study looked at the potential maximum amount of conversion from NH_4^+ to NO_3^- achievable if wastewater was aerated before injection into the subsurface (Appendix A). Each salinity treatment also had a planted control that received no wastewater to compare wastewater treated columns to control columns receiving only the corresponding saltwater. Treatment combinations were duplicated and randomly placed for a total of 16 wastewater-treated columns and 4 control columns.

Columns were made from 15.24 cm (inner diameter) PVC pipe. Four columns were made from clear PVC pipe to allow for viewing of soil, but were covered with aluminum foil to prevent light from entering the subsurface and contributing to algal or microbial growth. The bottom of each column (Figure 4.3) was fitted with a distribution plate to allow for even flow of

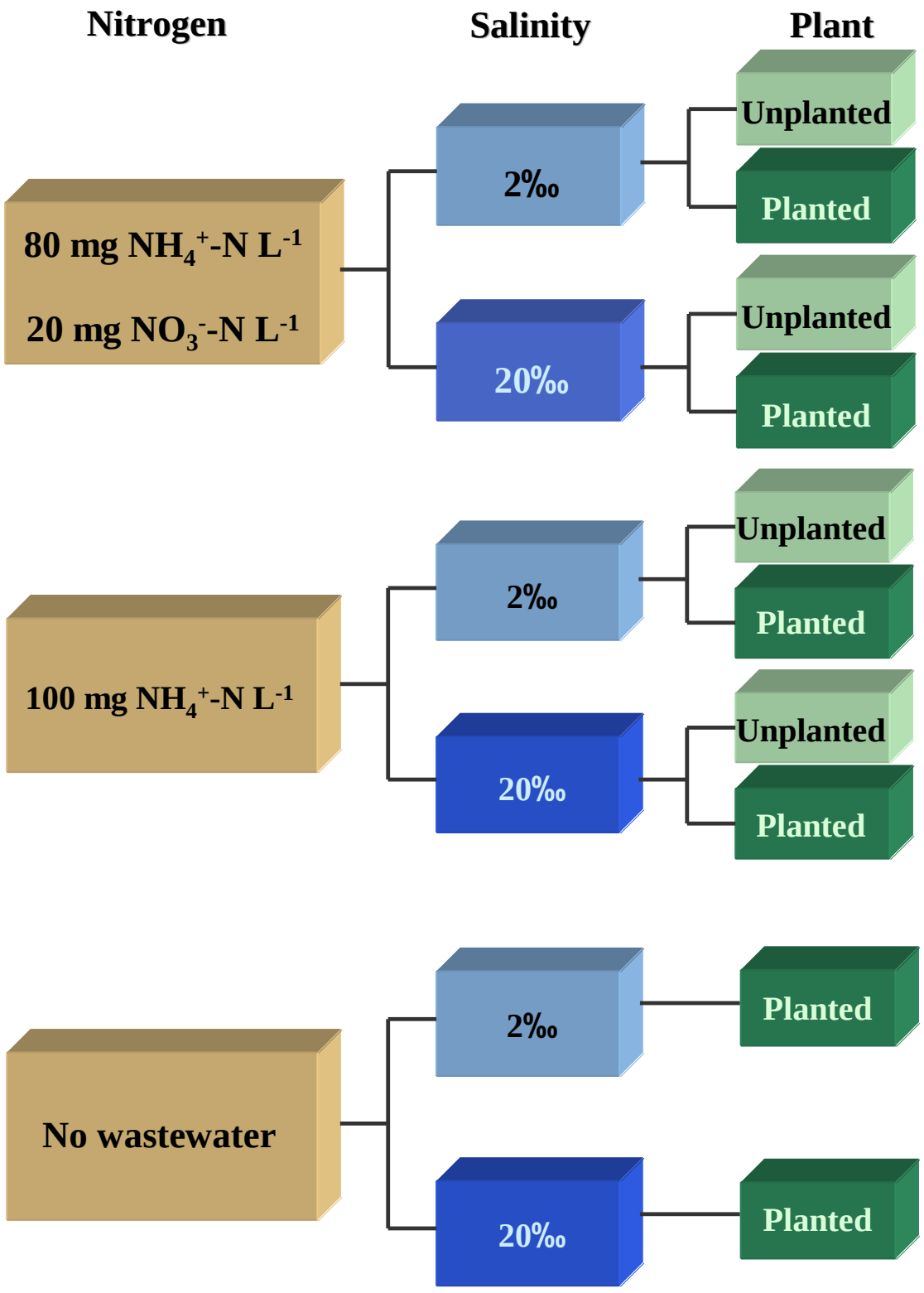


Figure 4.2 Layout of experimental design showing three treatments used. Each treatment combination was duplicated for a total of 20 experimental units (or columns). Parts per thousand is abbreviated as ‰.

the incoming wastewater as it passed through the gravel and sand layers. This ensured the entire bottom surface of the soil received the wastewater homogenously. Sampling ports were installed at 0, 8, 34, 58, and 84 cm below the surface of the soil. On the opposite side of the column from sampling ports, septa were installed to allow for measurement of redox potential. Platinum wire pieces of 2.54 cm were inserted through septa to permit redox potential readings to take place within the subsurface. The bottoms of the columns were filled with 7.6 cm of gravel and 7.6 cm of sand to prevent the distribution plate from clogging with the clay and silt present, as the holes in the distribution plate were approximately 0.16 cm in diameter.

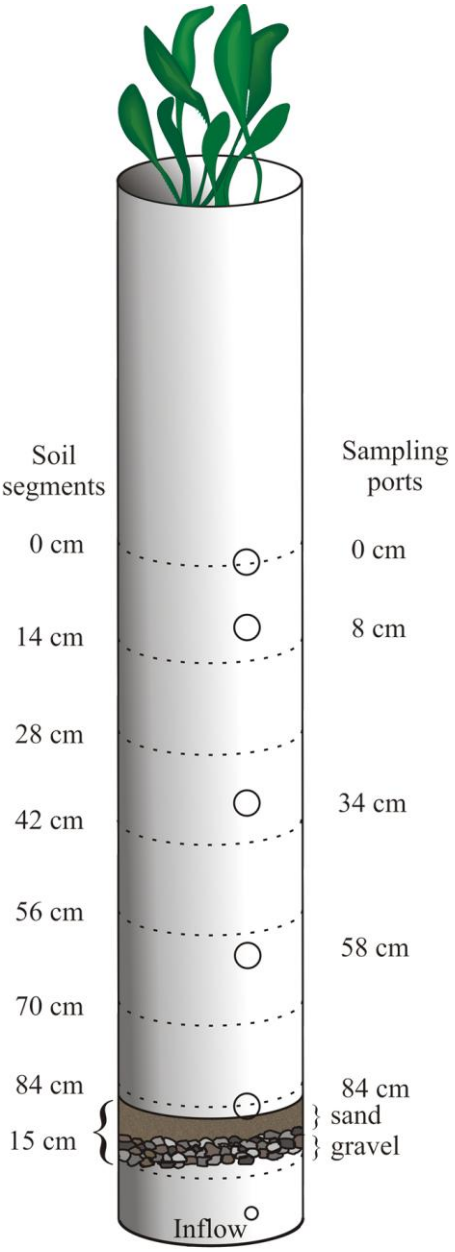


Figure 4.3 Schematic of column setup. Sampling port depths are indicated on right. Soil segments cut from column at end of experiment are indicated on left. The distribution plate was installed beneath gravel.

Soil collected from a salt marsh in Port Fourchon, Louisiana was homogenized, sterilized, and placed in each column up to a depth of 69 cm. In order to ensure measured microbial activity was due to microbes native to a particular salinity, soil was sterilized. Soil sterilization prevented introduction of saltmarsh microorganisms into the columns under the low salinity treatment, as soil was originally obtained from a salt marsh. Additional soil containing a higher amount of organic matter was collected from a salt marsh in Port Fourchon, Louisiana, and an intermediate marsh near Westwego, Louisiana, and was homogenized before adding to the columns to bring the total soil height to 84 cm. This organic matter was

added to columns of the corresponding salinity. The addition of the organic matter to the surface was done in order to achieve a soil profile more closely resembling the soil profile seen at field sites.

Saltwater of the target salinity was flushed through the columns until they reached the target salinity for their respective treatments. The columns were then inoculated with microorganisms from the appropriate marsh salinity. Microorganisms were extracted from the soil following the procedure by Riis et al., (1998). The procedure was only followed to step one as subsequent steps were for the isolation of microorganisms, which was not desired in this experiment. After centrifugation, the microorganisms were resuspended in 2.3 L of a 0.2% $Na_2P_2O_7$ solution and injected into the bottom four sampling ports. Columns receiving the planted treatment were planted with *Paspalum vaginatum* and *Spartina alterniflora* according to their native salinities, 2 and 20‰, respectively. An inert plastic mesh (~ 0.32 cm) was placed at the interface (15 cm below the surface) between the subsurface soil and higher organic matter surface soil to prevent roots from growing below that depth. Light banks were installed and maintained at approximately 200 μmol s^{-1} m^{-2} for approximately 12 hours each day to ensure plant growth was not inhibited by lack of light.

Standard synthetic wastewater (ASTM, 2006) was used with the following amendments: salt, nitrogen, and phosphorus levels were increased to the desired wastewater concentrations, kaolin (a mineral) was not added, a supplement (Reef Plus, Aquatic Ecosystems) containing trace nutrients was added, and the amount of beer (carbon source) was decreased from 6% to 1%. The amount of beer was decreased because initial tests found the dissolved organic carbon (DOC) level to be too high in comparison to field wastewater levels. Wastewater-treated columns received 12 mg PO_4^{3-}-P L^{-1} and 220 mg C L^{-1}. Saltwater solutions were made up using individual components (Table 4.1) (Atkinson and Bingman, 1998) as opposed to a commercial

Table 4.1 Concentrations of salts used in synthetic wastewater for the 2 and 20‰ and 100 mg NH_4^+-N L^{-1} (A) and 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N) treatments.

	Treatment			
	2A	2N	20A	20N
Salts	Concentration (g L^{-1})			
NH_4Cl	0.38	0.30	0.38	0.30
KNO_3	--	0.15	--	0.15
$Na_3PO_4 \cdot 12H_2O$	0.18	0.18	0.18	0.18
$NaHCO_3$	0.25	0.25	0.25	0.25
$MgCl_2 \cdot 6H_2O$	0.62	0.62	6.25	6.25
$CaCl_2 \cdot 2H_2O$	0.09	0.09	0.85	0.85
KCl	0.04	--	0.29	0.29
NaCl	0.62	0.20	16.00	15.80
K_2SO_4	0.002	0.002	0.02	0.02

ocean salt mix because initial tests found the sulfide levels (from reduction of seawater sulfate) to be extremely high in some columns (22 mM S^{2-} in one column), to the point of toxicity to plant growth. Sulfate is reduced to sulfide under strongly reduced conditions, such as occurred in this study. Koch and Mendelssohn (1989) found that just 1 mM S^{2-} significantly decreased the growth of two marsh plants. Field studies of the MUS have shown very little sulfide exists within the MUS wastewater plume, justifying the reduction of sulfur levels to 0.1 (20‰) and 0.01 (2‰) mg SO_4^{2-}-S L^{-1}.

Two L of synthetic wastewater were pumped into the bottom of each column every other day, equivalent to an injection flow rate of 0.03 L min^{-1}. The addition of wastewater took approximately an hour, which was equal to a mean flowrate of 0.7 mL min^{-1} over two days and led to a calculated complete turnover of wastewater within the column porewater approximately every eight days. Wastewater was sampled at every addition which was every two days. Samples were collected from each sampling port (before wastewater addition) on days 0, 5, 14, and every 14 days thereafter for a total of 84 days. Salinity, pH, and temperature of the samples

were measured at time of collection. Redox potential of the soil was also measured on the collection day using a SCE reference electrode pushed into the surface of the columns along with the platinum wire inserted through the septa. Surface redox potential was measured using a platinum electrode inserted into the soil to a depth of 5 cm. All redox measurements (E_c) were corrected to a standard hydrogen reference electrode and expressed as E_h. At the end of the study, columns were cut into seven sections as shown in Figure 4.3. Each section was then mixed to ensure a homogenous mixture of sediment and stored at 4°C until further analysis.

4.2.2 Water Analyses

Samples were filtered (0.45 μm) and measured for nitrate + nitrite (NO_3^- + NO_2^-, or NO_X, EPA method 353.2), soluble reactive phosphorus (SRP, EPA method 365.1), ammonium (NH_4^+, EPA method 350.1) (USEPA, 1993), total phosphorus (TP, method 4500-P B and E) (APHA, 1998), and DOC. Samples were preserved at pH < 2 and stored at 4°C until analysis took place except NO_X, which was analyzed unacidified within 48 hours of sampling. Ammonium, NO_X, and SRP were measured using a Seal Analytical AQ2 Automated Discrete Analyzer (Mequon, WI). Dissolved organic carbon was measured directly using a Shimadzu TOC-V_{CSN} analyzer (Columbia, MD).

4.2.3 Soil Analyses

Sand, silt, and clay fractions were measured using sieve (ASTM C117, C136) and hydrometer analyses (ASTM D422) (ASTM, 1995). X-ray diffraction analysis was performed in the Louisiana State University Department of Geology X-ray crystallography laboratory using a Bruker/Siemens D5000 automated powder X-ray diffractometer (Madison, WI) with Rietveld analysis software. Pre-study, soils were measured for organic matter (OM) content, percent moisture, and total carbon and nitrogen (TC and TN). Post-study, soils were measured for bulk density, organic matter (OM) content, percent moisture, and total carbon and nitrogen (TC and

TN). Bulk density was calculated for each soil segment, excluding the sand/gravel segment, on a dry weight basis. Organic matter was measured as loss on ignition at 435°C (Sparks, 1996). Percent moisture was calculated (as g of water in soil sample * 100/g dry soil in sample) on ~ 10 g soil dried at 70°C until constant weight (Klute, 1986). Total C and TN were determined on dried, ground samples using a Costech Elemental Combustion System (Valencia, CA). Exchangeable NH_4^+ retained in the soil at the end of the study was extracted following Mulvaney (1996) with the following amendments: 25 mls of 2M KCl was added to ~ 2 g wet soil in a 30-ml centrifuge tube and shaken for 30 min. Exchangeable NH_4^+ was measured following the same procedure as the water samples. Cation exchange capacity was determined following Sparks (1996).

4.2.4 Statistical Analyses

SAS® software (2009) and SigmaPlot® software (2002) were used to analyze the data. All statistical tests were performed at a significance level of $\alpha = 0.05$. A three-way ANOVA (between salinity, plants, and nitrogen) with a split-plot addition for depth and time was applied to compare treatments. Controls were excluded from the three-way ANOVA as they led to an unbalanced design. In order to compare controls to wastewater treated columns, comparisons to control data were made using a one-way ANOVA (using the 10 treatment combinations) with linear combinations between controls and wastewater treated columns. A one-way ANOVA was used to analyze influent versus effluent differences. Influent and column means (± standard deviation or SD) were calculated for the entire length of the study. Treatment efficiencies were calculated using the influent and surface sampling port data. Ammonium percent removal was calculated by subtracting the effluent (at the surface) from the influent and then dividing by the influent. The percent removal was then used to find the removal rate by plotting the removal

rate for each sample date and then finding the slope of the line for each time frame (Days 0 – 14, 14 – 56, 56 – 84). Removal rates were then compared using a one-way ANOVA.

4.3 Results and Discussion

4.3.1 Soil Characteristics

The top 15 cm of soil in each column contained OM obtained from a marsh of a similar salinity in order to better simulate field sites. Organic matter content for the low salinity soil was 77%, while OM content for the high salinity was only 24%. Both OM layers were found to be a silt loam soil. A sandier soil collected for the main part of the column (15 – 84 cm below the surface) contained only 1.3% OM. The sandy soil was found to be a loamy sand with a CEC of 14.5 centimoles of cation charge kg-soil^{-1} (Table 4.2). This CEC falls within the range of other wetland soil CECs ranging from 4.4 to 130 centimoles of cation charge kg-soil^{-1} (Hou et al., 2000; Chen et al., 2002; Johns et al., 2004).

At the end of the study, the columns were sectioned for a total of 120 soil samples and 20 sand/gravel samples from the bottom of each column. Analysis of soil data found, for all parameters measured (excluding bulk density), the mean was higher at the surface and decreased with depth (Table 4.3). Bulk density showed the opposite trend and increased with depth (Table

Table 4.2 Percent sand, silt, and clay and initial mean organic matter content, CEC, total carbon, and total nitrogen for soil used in columns.

	cm below surface		
	0 - 15		15 - 84
Property	20‰	2‰	all
sand (%)	13	22	78
silt (%)	72	58	18
clay (%)	15	20	4
USDA classification	silt loam	silt loam	loamy sand
organic matter (%)	24	77	1.3
CEC (centimoles of cation charge kg-soil^{-1})	-	-	14.5
total carbon (g C kg soil^{-1})	-	-	10
total nitrogen (g N kg soil^{-1})	-	-	BDL[a]

[a]Below detection limit of 0.5 g N kg-soil^{-1}.

4.3). There were no significant differences seen in the soil sections from 28 – 84 cm and the sand section (below the surface). Therefore, these data are averaged together for tabular presentation in order to make the data more clear. The top 15 cm of soil in each column contained OM obtained from a marsh of a similar salinity in order to better simulate field sites, thus from 0 – 14 cm the soil was entirely comprised of high OM material and from 14 – 28 cmthe soil was a mixture of the surface OM layer and the soil found from 28 – 84 cm. This accounted for the mid-range values found from 14 – 28 cm for most parameters (Table 4.3).

Organic matter content at the end of the study ranged from 1.6 – 62%, with the lowest percentages found in the subsurface (28 – 84 cm) (Table 4.3). Significance was found for the salinity*plants*nitrogen*depth interaction ($p < 0.05$). The significance in OM content was only found from 0 – 14 cm, where both of the low salinity, unplanted treatments had a significantly higher OM content relative to all other treatments at this depth (Figure 4.4). The initial percentages of OM account for the significant differences seen in OM. Initially, the OM content of the low salinity treatment was 77%, while the high salinity treatment was 24%. The initial difference in OM content was because of the initial setup where soil collected from marshes of similar salinities was added to better simulate the OM content of natural wetlands in previous MUS field sites.

The surface OM content decreased from 77% to 32 (± 25%) and from 24% to 17 (± 5%) for the low and high salinity treatments, respectively, during the course of this study (Tables 4.2 and 4.3). This was likely due to microbial processes that consumed the readily available organic carbon present. In contrast to this decrease, the subsurface increased from 1.3% to a mean of 2.5 (± 0.9%). The weight of the soil from 28 – 84 cm was used, along with the amount of DOC added at a flow rate of 220 mg DOC d^{-1} for 133 days (the length of time before the columns were sectioned), to find the total loading of DOC. The total loading of DOC) from 28 – 84 cm was

Table 4.3 Mean soil percent moisture, organic matter content, bulk density, and total carbon and nitrogen by depth for control and wastewater treated columns at the end of the study. The 28 – 84 cm depth includes the sand layer, except for bulk density, for which it was not measured.

		Parameter				
Treatment	Depth (cm)	moisture (%)	organic matter (%)	bulk density (g cm^{-3})	total carbon (g C kg $soil^{-1}$)	total nitrogen (g N kg $soil^{-1}$)
20PC	0 - 14	118 ± 7.7	10 ± 2.0	0.49 ± 0.11	31 ± 11	1.9 ± 0.6
	14 - 28	69 ± 21	4.7 ± 0.02	0.84 ± 0.11	15 ± 2.0	0.91 ± 0.21
	28 - 84	27 ± 3.2	2.2 ± 0.6	1.48 ± 0.04	6.3 ± 1.0	BDL[a]
2PC	0 - 14	186 ± 50	10 ± 0.7	0.18 ± 0.21	61 ± 13	4.0 ± 0.7
	14 - 28	78 ± 12	3.0 ± 0.9	0.72 ± 0.14	13 ± 1.3	0.84± 0.16
	28 - 84	28 ± 3.9	1.6 ± 0.7	1.47 ± 0.05	6.3 ± 0.9	BDL
20PN	0 - 14	195 ± 63	19 ± 4.3	0.28 ± 0.04	83 ± 27	5.2 ± 0.8
	14 - 28	58 ± 1.9	4.5 ± 0.6	0.95 ± 0.02	17 ± 1.1	1.2 ± 0.2
	28 - 84	26 ± 4.3	2.1 ± 0.6	1.44 ± 0.04	6.4 ± 2.0	BDL
2PN	0 - 14	156 ± 10	11 ± 0.9	0.39 ± 0.02	46 ± 4.7	5.1 ± 0.2
	14 - 28	85 ± 1.8	4.3 ± 3.3	0.67 ± 0.001	18 ± 16	0.97 ± 1.4
	28 - 84	26 ± 4.7	2.1 ± 0.7	1.50 ± 0.07	5.9 ± 1.7	BDL
20UN	0 - 14	246 ± 26	20 ± 1.9	0.20 ± 0.08	80 ± 0.7	5.8 ± 0.06
	14 - 28	77 ± 41	6.6 ± 3.8	0.86 ± 0.34	23 ± 13	1.6 ± 1.1
	28 - 84	26 ± 5.1	2.0 ± 1.0	1.45 ± 0.05	6.4 ± 2.0	BDL
2UN	0 - 14	833 ± 34	62 ± 2.7	0.07 ± 0.02	301 ± 5.6	22 ± 0.7
	14 - 28	108 ± 18	9.0 ± 0.1	0.57 ± 0.13	42 ± 12	3.1 ± 0.7
	28 - 84	26 ± 4.1	2.1 ± 0.9	1.47 ± 0.11	5.8 ± 1.6	BDL
20PA	0 - 14	104 ± 6.3	9.8 ± 2.0	0.52 ± 0.06	38 ± 2.7	2.4 ± 0.4
	14 - 28	55 ± 9.3	4.5 ± 1.1	0.96 ± 0.14	14 ± 4.6	0.89 ± 0.27
	28 - 84	26 ± 3.6	3.3 ± 1.2	1.45 ± 0.05	6.5 ± 0.9	BDL
2PA	0 - 14	141 ± 6.2	9.8 ± 0.2	0.38 ± 0.05	46.4 ± 9.9	3.4 ± 0.8
	14 - 28	78 ± 12	5.9 ± 0.3	0.77 ± 0.06	25 ± 5.1	1.8 ± 0.2
	28 - 84	26 ± 4.8	2.2 ± 0.9	1.44 ± 0.06	6.2 ± 1.6	BDL
20UA	0 - 14	222 ± 17	18 ± 2.7	0.22 ± 0.001	77 ± 13	5.6 ± 0.7
	14 - 28	73 ± 16	4.5 ± 1.7	0.82 ± 0.16	19 ± 0.1	1.1 ± 0.2
	28 - 84	24 ± 5.5	2.7 ± 1.0	1.47 ± 0.06	6.1 ± 2.0	BDL
2UA	0 - 14	564 ± 177	46 ± 15	0.10 ± 0.04	225 ± 87	17 ± 6.2
	14 - 28	87 ± 33	6.4 ± 1.9	0.72 ± 0.24	28 ± 7.8	2.0 ± 0.9
	28 - 84	26 ± 5.1	1.6 ± 1.0	1.44 ± 0.08	4.9 ± 2.6	BDL

[a]Below detection limit of 0.5 g N kg-$soil^{-1}$.

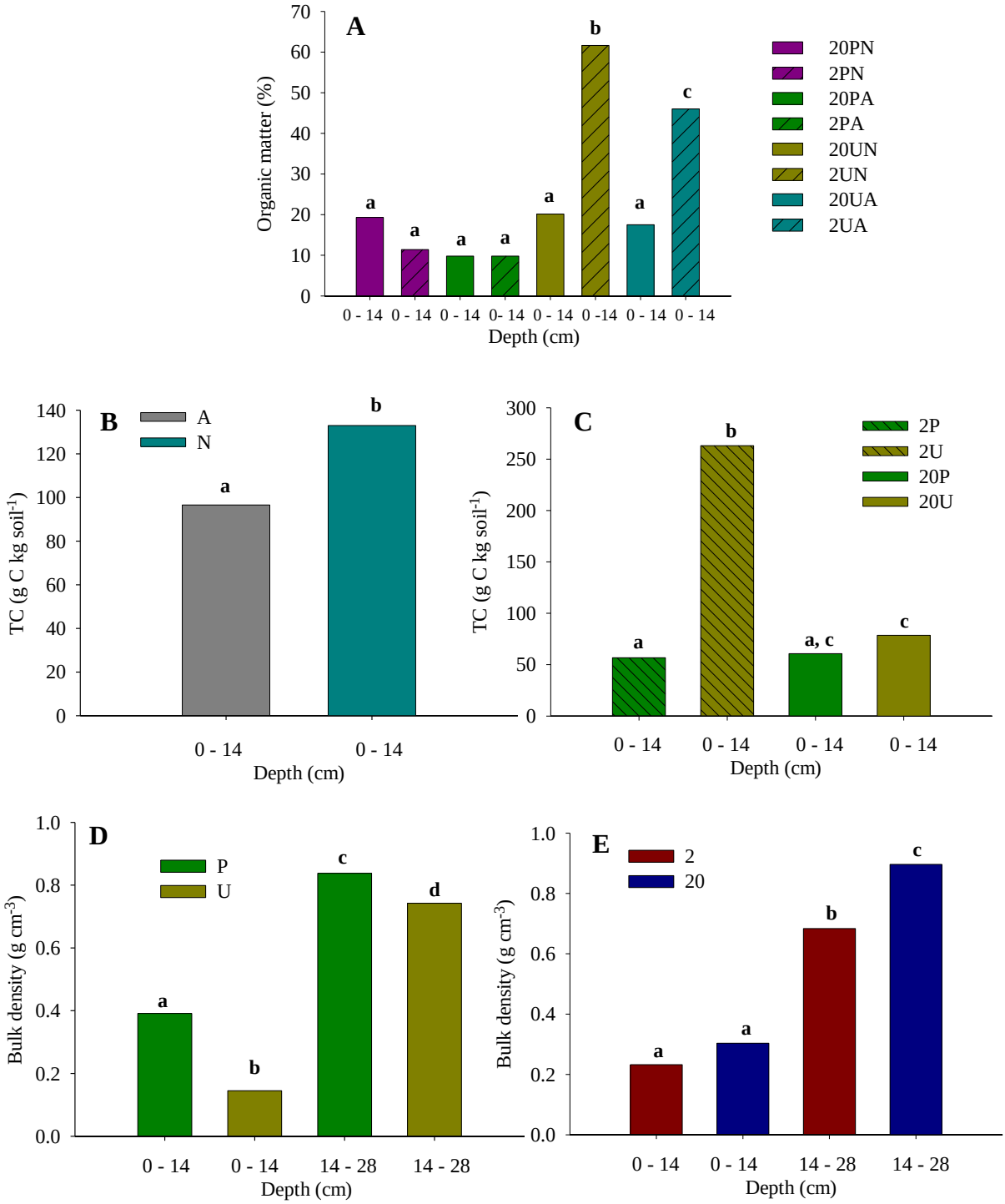


Figure 4.4 Significant statistical comparisons of soil characteristics. Letters indicate treatment combinations with significant differences. Treatments are not compared to controls as comparison to controls was done with a different analysis. Only significant depths are shown, all depths not shown were found to have no significant difference. Graph A shows organic matter content, B and C show total carbon (TC), and D and E show bulk density.

1.5 g C kg soil^{-1} or 0.15% DOC, which would not account for the estimated increase of 0.6% DOC found within the columns. A previous experimental trial was run on the columns, with wastewater having a higher DOC content, and possibly accounted for some of the increase in OM content.

Total carbon ranged from 5 – 301 g C kg soil^{-1} (Table 4.3). Significant interactions in TC were found for nitrogen*depth and salinity*plants*depth ($p < 0.001$). For the nitrogen*depth interaction, at a depth of 0 – 14 cm the 80%NH_4^+/20%NO_3^- treatment had a higher TC than the 100%NH_4^+ treatment (Figure 4.4). However, there was no significant effect of nitrogen at any other depth. From 0 – 14 cm, the low salinity, unplanted treatment had a significantly higher TC than all other salinity*plant combinations. At this depth, TC was dependent on both the salinity level and the presence or absence of plants (Figure 4.4). The significance of the salinity*plant combination was also dependent on the initial percentages of OM in the soil, as the low salinity treatment had a higher level of OM, which would contribute to a higher level of TC. Total C was significantly higher ($p < 0.001$) in wastewater treated columns versus control columns, an indication addition of wastewater increased TC levels.

Total N was only measurable from 0 – 28 cm and ranged from 0.89 – 22 g N kg soil^{-1} (Table 4.3). All other depths were below the detection limit of 0.5 g N kg soil^{-1}. Significant interactions in TN were found for salinity*plants*depth ($p < 0.001$). From 0 – 14 cm, only the low salinity, unplanted treatment was significant. This treatment had a significantly higher TN relative to all other treatments. Though the higher levels of OM in the low salinity treatments likely account for the higher TN from 0 – 14 cm, sorption of wastewater nitrogen to OM may also have contributed to the higher TN concentrations at this depth.

Bulk density averaged 1.46 (± 0.07) g cm^{-3} from 28 – 84 cm, 0.79 (± 0.18) g cm^{-3} from 14 – 28 cm, and 0.27 (± 0.15) g cm^{-3} for 0 – 14 cm below the surface (Table 4.3). Both the

salinity*depth and plant*depth interactions were significant ($p < 0.001$). From 0 – 14 cm and 14 – 28 cm, the bulk density was significantly lower in the unplanted treatment versus the planted treatment. Bulk density was significantly lower in the low salinity treatment than the high salinity at the same depths (Figure 4.4). This was likely due to the differences in OM content of the surface soil applied to simulate field conditions. There were no significant differences in the bulk density from 28 – 84 cm. Bulk density would normally be expected to be lower in the planted columns, as plant roots (and therefore OM) lower bulk density (DeLaune et al., 1979; Baustian et al., 2009). However, the unplanted columns in this study had a higher OM content. The lower bulk density in unplanted columns and in the low salinity was explained by the presence of microbial mats that formed near the surface (0 – 28 cm) during the experiment. The microbial mats were most prevalent in the low salinity, unplanted columns and were very spongy and rose to an approximate height of 12 cm above the soil surface. Microbial mats are composed of a complex consortium of cohesive bacteria and are responsible for a wide variety of biochemical and ecological interactions. They are ubiquitous and found from marine and freshwaters to wetlands to soil to deep ocean hydrothermal vents and Antarctic ponds and sea ice (Bender and Phillips, 2004; Rejmankova and Komarkova, 2005). The wide ranging redox gradients present in most mats help account for the high productivity seen in some mats (Bender and Phillips, 2004). The buoyancy often exhibited by microbial mats is due to the formation of gases within their matrix, which explained the increase in soil height and lower bulk densities seen in the low salinity, unplanted columns in this study.

4.3.2 Overall Analysis

A total of 206 wastewater samples and 800 column water samples were collected over the course of 84 days. Each column received a total of 76 L of wastewater over the course of the study, which equaled approximately eleven porewater exchanges. *In situ* parameters (i.e.,

temperature, salinity, pH, and redox potential) and nutrient levels were measured in the influent wastewater (Table 4.4). Mean levels were fairly consistent throughout the study for most parameters. The presence of NO_X in the 100 NH_4^+-N L^{-1} and control (no wastewater) treatments is likely due to some salt contamination or some nitrification occurring in the 100 NH_4^+-N L^{-1} treatment.

Data were consolidated when no significant differences were found between treatments for *in situ* parameters from wastewater treated columns. Overall wastewater column means for temperature, pH, and redox potential were 22.4 (± 1.0)°C, 6.83 (± 0.36), and -87 (± 117) mV, respectively. There was no significant difference between treatments for temperature, pH, or

Table 4.4 Overall mean of influent parameters. Treatments are abbreviated as 100 mg NH_4^+-N L^{-1} (A) vs. 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N), and control (planted columns receiving no wastewater, C).

	Treatment					
Parameter	2A	2N	2C	20A	20N	20C
DO	7.89 ± 0.22	7.86 ± 0.27	8.08 ± 0.09	7.12 ± 0.16	7.19 ± 0.17	7.30 ± 0.07
(mg L^{-1})	(27)	(27)	(25)	(27)	(27)	(25)
Salinity	2.1 ± 0.2	1.9 ± 0.3	1.9 ± 0.4	21.6 ± 2.1	21.7 ± 2.1	23.7 ± 2.2
(‰)	(43)	(43)	(16)	(43)	(43)	(16)
pH	7.94 ± 0.42	7.98 ± 0.33	7.81 ± 0.24	7.76 ± 0.38	7.79 ± 0.35	7.84 ± 0.13
	(43)	(43)	(16)	(43)	(43)	(16)
Temperature	22.1 ± 1.4	22.0 ± 1.6	22.7 ± 1.3	22.1 ± 1.7	22.1 ± 1.6	22.4 ± 1.2
(°C)	(43)	(43)	(17)	(43)	(43)	(17)
DOC	232 ± 58	218 ± 58	35 ± 14	217 ± 56	214 ± 56	29 ± 21
(mg C L^{-1})	(36)	(36)	(12)	(36)	(36)	(11)
NH_4^+	91 ± 7.5	73 ± 7.5	0.46 ± 0.60	97 ± 7.1	77 ± 7.1	0.77 ± 0.82
(mg N L^{-1})	(43)	(43)	(16)	(43)	(43)	(15)
NO_X	0.08 ± 0.05	18 ± 2.4	0.05 ± 0.04	0.16 ± 0.35	18 ± 2.0	0.07 ± 0.11
(mg N L^{-1})	(43)	(43)	(16)	(43)	(43)	(16)
SRP	14 ± 2.6	14 ± 1.9	0.05 ± 0.11	14 ± 2.2	14 ± 1.8	0.09 ± 0.14
(mg P L^{-1})	(43)	(43)	(16)	(43)	(43)	(16)

n=( ).

redox potential (Figure 4.5) in wastewater treated columns. Salinity in the columns averaged 23.0 (± 3.8) and 3.1 (± 3.4)‰ for the 20 and 2 treatments, respectively and was significantly different ($p < 0.001$) between the low and high salinity treatments. The low salinity treatments had a mean of 3‰ due to a brief influx of the high salinity influent in one of the low salinity columns that was rectified as soon as it was discovered. Redox potential, pH, and salinity remained relatively constant throughout the course of the study after an initial period of acclimation to the wastewater (Figure 4.5). Temperature remained constant throughout the course of the study, excluding day 0. The temperature in the laboratory on day 0 was significantly higher due to a malfunctioning of the building's cooling system. Time was found to be significant ($p < 0.01$) for all parameters and was due to the initial acclimation of the wastewater, except for temperature which was due to the malfunctioning cooling system. Influent salinity and temperature means were not significantly higher than overall column means. The overall mean redox potential moved towards a mean of approximately -100 mV by the end of the study (Figure 4.5). Redox potential was found to be significant for depth ($p < 0.01$). This significance is accounted for by the surface (-5 cm) and bottom-most soils (-84 cm) (Figure 4.6), which were more oxidized and, thus, had a higher redox potential than the soil layers in between (-8 to -58 cm). The bottom-most soils were more oxidized because of their proximity to the incoming wastewater, which had a mean DO level of 7.57 mg L^{-1}. As wastewater moved further up the column, the available oxygen was less, as it was consumed in deeper soils. The mean redox potential of -118 mV indicates that sulfur reduction was most likely to occur (-100 to -200 mV) (Reddy and DeLaune, 2008). However, available sulfur was also decreased in the saltwater mixture to more closely match concentrations seen in the field, so sulfur reduction was not likely to be the primary microbial process occurring. Nitrate reduction generally occurs around 250 mV, but the actual process occurring at a specific redox potential would be dependent on many

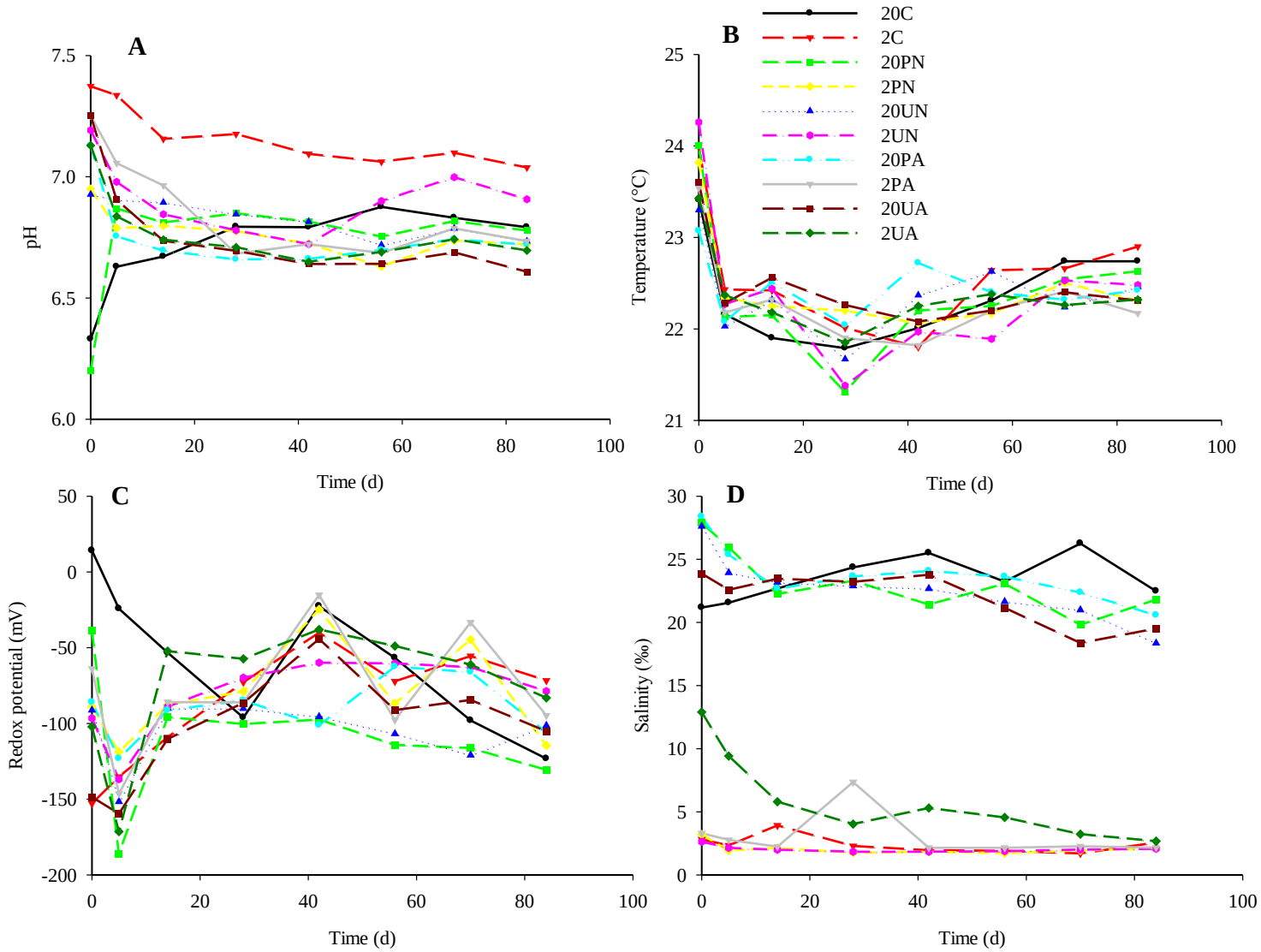


Figure 4.5 *In situ* parameters for individual treatment combinations averaged over the whole column. Graph A shows pH, B shows temperature, C shows redox potential, and D shows salinity. Treatments are abbreviated as planted (P) vs. unplanted (U), 100 mg NH_4^+-N L^{-1} (A) vs. 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N), and control (planted columns receiving no wastewater, C).

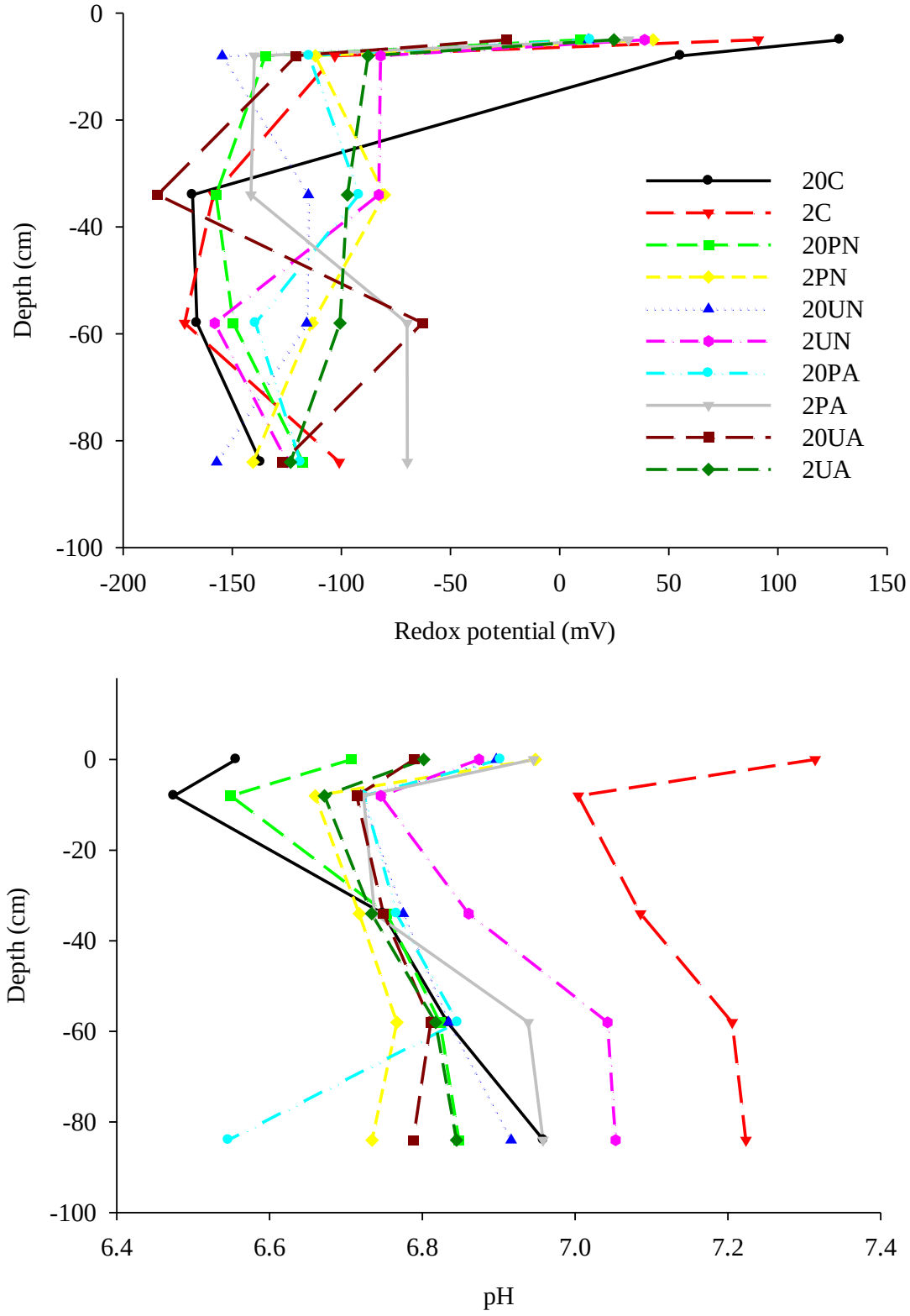


Figure 4.6 Redox potential and pH by depth for individual treatment combinations averaged over the entire study. Treatments are abbreviated as planted (P) vs. unplanted (U), 100 mg NH_4^+-N L^{-1} (A) vs. 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N), and control (planted columns receiving no wastewater, C).

environmental factors, including the pH and availability of carbon and electron acceptors (Reddy and DeLaune, 2008; Rivett et al., 2008).

Influent pH was significantly higher ($p < 0.001$) than overall column means and dropped from a mean of 7.87 (± 0.38) to an overall column mean of 6.80. The drop in pH was due to the reducing conditions of the soil, as pH tends toward neutrality under reducing conditions (Patrick et al., 1996). Wastewater treated columns also had a significantly lower pH ($p < 0.001$) than control columns with an overall column mean of 6.94 (± 0.49). The lower pH found in wastewater treated columns was likely due to an increase in organic and carbonic acids as the wastewater was broken down, which could also explain the lower pH found in columns versus the influent. The salinity*plants*days and salinity*nitrogen*days interactions were found to be significant ($p < 0.05$) for wastewater treated columns. These interactions were significant due to the low pH of treatment 20PN on day 0 (Figure 4.5). The pH for all columns, however, moved towards neutrality, coming to a steady (and not significantly different) state between 6.6 and 7.1 by the end of the study (Figure 4.5). The surface was found to have a significantly higher ($p < 0.01$) pH than the subsurface (-5 cm to -84 cm). The surface would be expected to have a higher pH, as the surface was more oxidized (37 ± 100 mV) than the subsurface (-118 ± 100 mV). Surface pH was measured from the wastewater samples taken from the top port, while the surface redox potential was measured 5 cm below the top of the soil. The pH also increased with increasing depth from -8 to -58 cm, but from -58 to -84 cm the pH was nearly the same for most columns (Figure 4.6). The more highly reduced soils from -5 to -84 cm explained why the pH increased between these depths, as reduced soils tend to stabilize near a neutral pH (Patrick et al., 1996). The near-neutrality of the pH is also noteworthy as most microorganisms have optimal activity at a neutral pH, specifically for denitrifiers, which have an optimal pH range of 6 – 8 (Paul and Clark, 1996).

Dissolved organic carbon was significantly reduced ($p < 0.001$) from an influent mean of 220 to an overall effluent mean of 22.7 (± 16.8) mg C L^{-1} from columns receiving wastewater. This drop in DOC is equivalent to a removal efficiency of 90%. The mean of control columns was 12.5 (± 7.3) mg C L^{-1} and was significantly lower ($p < 0.001$) than columns receiving wastewater (Figure 4.7). The total loading of DOC for the entire study period was only 840 mg C kg $soil^{-1}$. Even with this seemingly small amount of carbon added, microbial growth was increased by the increase in DOC (Chapter 5). Wastewater brings in additional carbon that microorganisms are able to capture and utilize to increase their population and biomass as well as residual soil organic matter. As the microbial population grows, the amount of carbon consumed increases, even in reduced environments where the utilization of carbon is slower (White and Reddy, 2001).

Among columns receiving wastewater, the salinity*depth and salinity*day interactions were significant ($p < 0.05$). The salinity*depth interaction was significant at the surface due to higher DOC levels found in the high salinity treatment relative to the low salinity treatment (Figure 4.7). This difference was likely due to the increased microbial activity seen in the low salinity columns. High salinities have been found to have a negative impact on microbial activity (Laura, 1977; Seitzinger et al., 1991; Rysgaard et al., 1999; Sardinha et al., 2003; Wichern et al., 2006; Yuan et al., 2007a). Four of the low salinity columns had a very active microbial mat at the surface (Chapter 5). The more active the microbial community, the more carbon will be utilized as microbes incorporate carbon into their cells. If microbes consume carbon, less carbon will be present in the effluent. Though the high salinity treatment had a negative impact on the DOC effluent at the surface, this impact was only short-lived (Figure 4.8). Only days 28 and 42 showed a significant difference between the salinity treatments. This temporary significance was likely due to the acclimation of the microbial population. As the

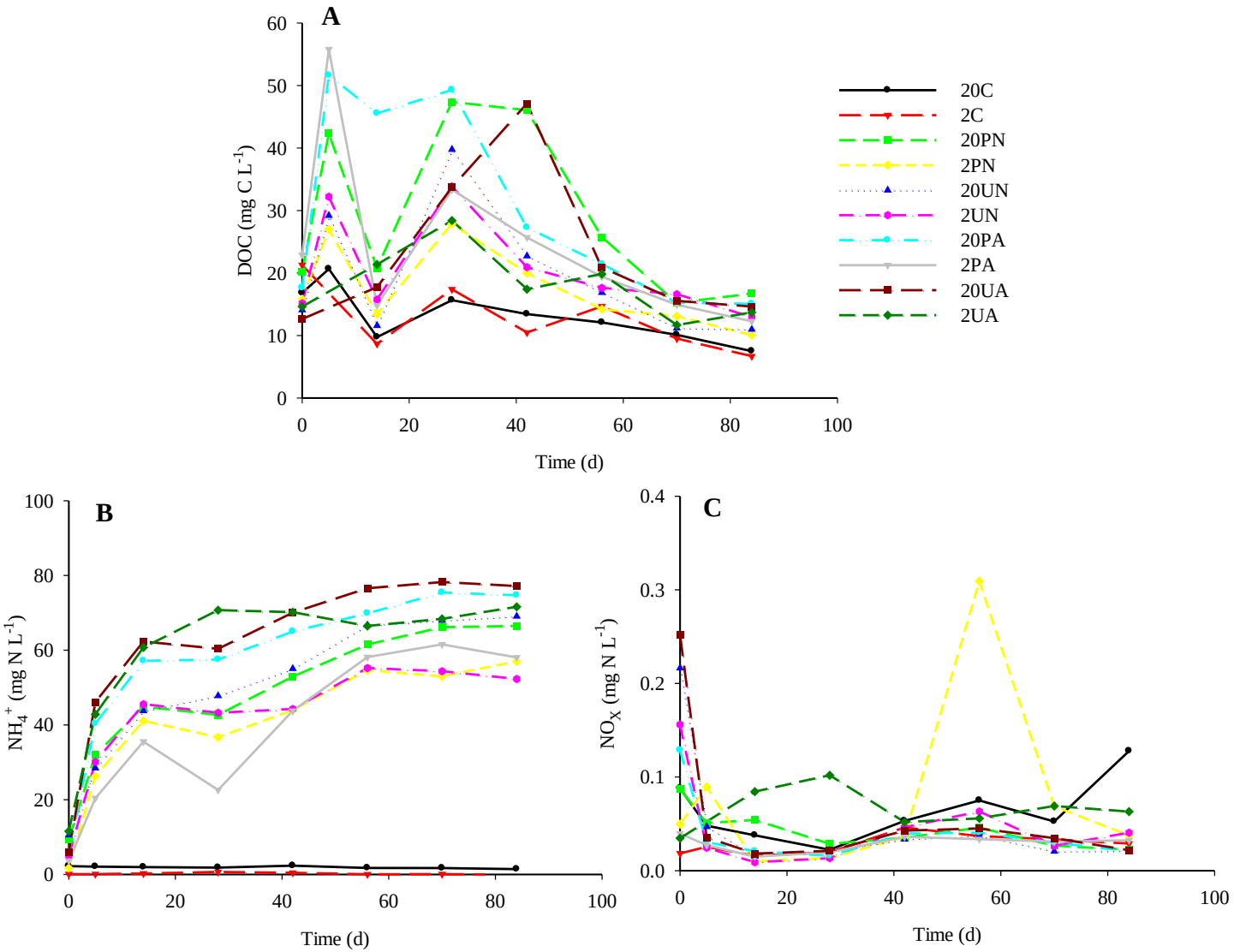


Figure 4.7 Dissolved organic carbon (graph A), NH_4^+ (graph B), and NO_X (graph C) for individual treatment combinations averaged over the whole column. Treatments are abbreviated as planted (P) vs. unplanted (U), 100 mg NH_4^+-N L^{-1} (A) vs. 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N), and control (planted columns receiving no wastewater, C).

high salinity (i.e., stress) killed off some microorganisms, those more able to cope with the high salinity conditions continued to grow and consume more carbon, thus reducing the DOC present within the effluent.

4.3.3 Nitrate Treatment

Nitrate was significantly ($p < 0.001$) reduced from an influent mean of 18.43 (± 2.18) to an overall effluent mean of 0.06 (± 0.15) mg NO_X-N L^{-1} in columns receiving NO_3^-. This led to removal efficiencies for NO_X that were upwards of 99%, within just a few cm from the influent, for the entire length of the study. Previous field studies of the MUS also found no detectable amounts of NO_X (Turriciano, 2005; Fontenot et al., 2006). In comparison, another laboratory constructed wetland treating synthetic wastewater saw nitrate removal efficiencies of 98% over the course of 20 months (Wiessner et al., 2005). Two natural, forested, treatment wetlands

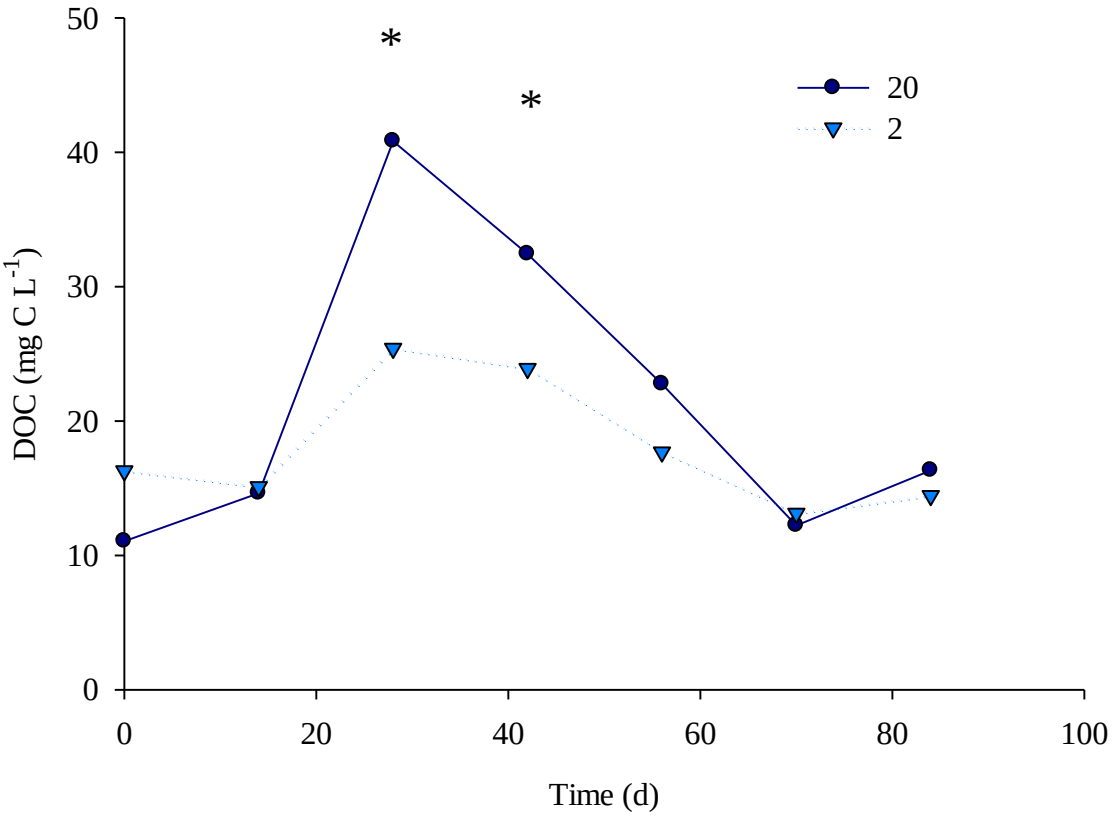


Figure 4.8 Mean dissolved organic carbon effluent (at surface) for the 2 and 20‰ salinity treatments. Stars indicate days on which there was a significant difference between salinities.

receiving wastewater from municipal sources had 100% removal efficiency (Day et al., 2004). A stormwater treatment wetland in Malaysia had a removal efficiency of 71% (Sim et al., 2008). Planted surface flow wetlands, treating nitrate-contaminated groundwater, had efficiencies ranging from 70 – 99% as compared to unplanted surface flow wetlands, from the same study, that had a 55% removal efficiency (Lin et al., 2007). Overall, this study, representing subsurface injection of wastewater, showed NO_X removal efficiencies similar to and higher than other treatment wetlands.

To compare effects on nitrate treatment, statistical analysis was run only on columns receiving nitrate. Among these columns, the plants*time interaction and depth were significant ($p < 0.05$). The plant*time interaction was significant due to higher nitrate levels present in the unplanted treatment on day 0. As wastewater introduction began and flooded conditions were sustained, the nitrate levels dropped and were no longer significantly different among treatments on later days. Nitrate levels were significantly higher at the surface relative to the subsurface (-8 to -84 cm). This is explained by the high levels of NH_4^+ received by the columns, which were likely converted to NO_3^- via nitrification at the more oxidized surface, thus accounting for the higher NO_X levels found at the surface.

Effluent concentrations (at all depths) of NO_X (Figure 4.7) showed no significant difference between nitrogen treatments (Figure 4.9, only data representative of all treatments is shown). Nitrate present in wastewater entering the columns from the bottom was consumed within a short distance as the effluent from the lowest sampling port (-84 cm) showed no difference among columns receiving NO_3^- versus columns not receiving NO_3^-. Disappearance of NO_3^- was most likely due to the occurrence of denitrification. The mean surface (-5 cm) redox potential was 37 and mean subsurface (-8 to -84) was -118 mV, both within the redox potential

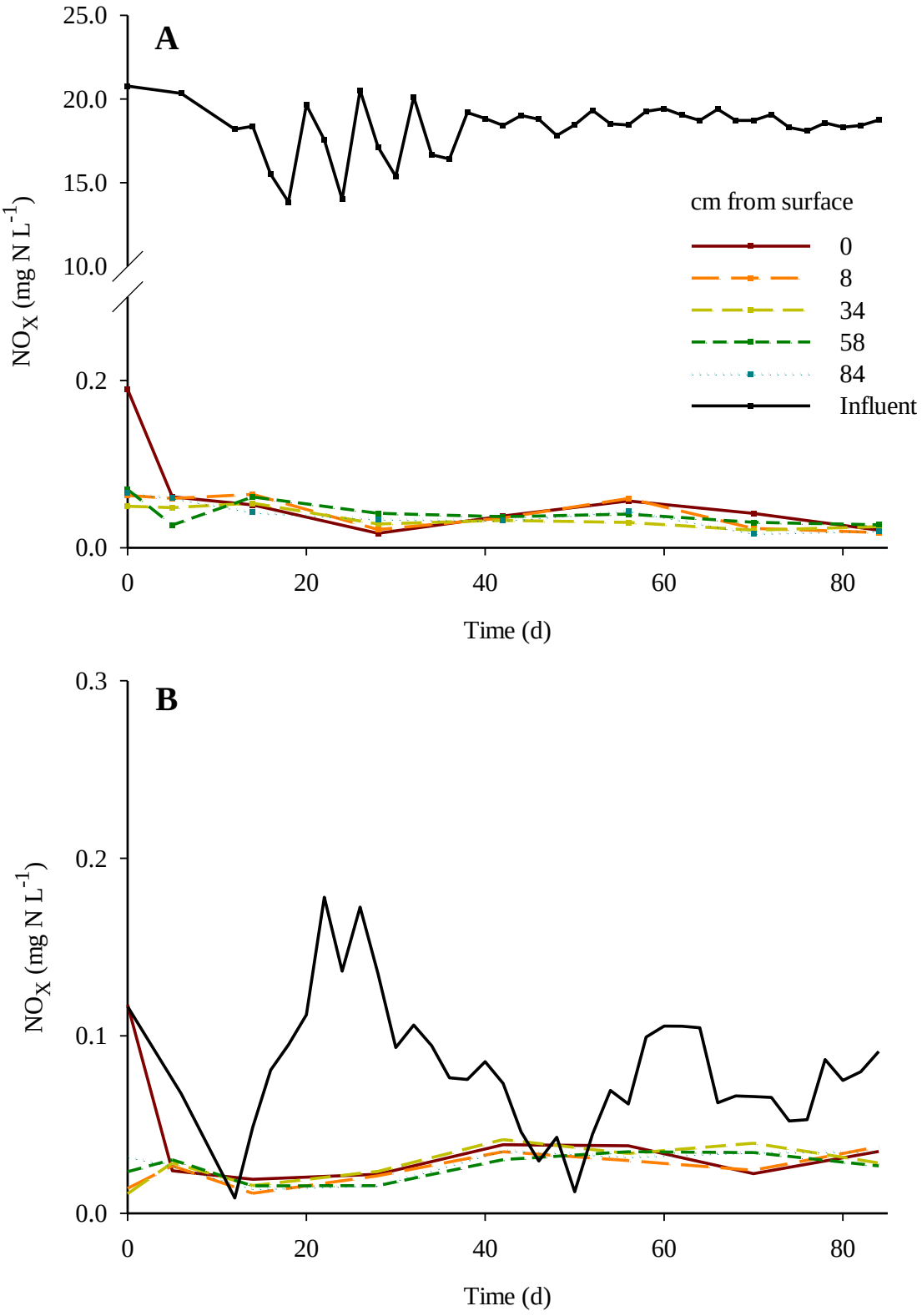


Figure 4.9 Representative graphs of NO_X versus time. Lines represent the mean. Graph A shows 20‰, planted, 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} treatment combination and Graph B shows 2‰, planted, 100 mg NH_4^+-N L^{-1}. Remaining graphs are presented in Appendix B.

range in which denitrification occurs. Though the average redox potential for initiation of denitrification occurs around 250 mV, redox potential for initiation of denitrification ranges from 100 to 300 mV (Reddy and DeLaune, 2008; Rivett et al., 2008). Additionally, the increased DOC levels due to wastewater would favor denitrifier activity.

Denitrification enzyme activity (DEA, discussed in Chapter 5) was performed on soil segments from 0 – 28 cm and in the bottom (70 – 84 cm/sand layer) where wastewater entered the columns. The presence of DEA within the bottom 14 cm of soil (only in columns which received nitrate), indicated nitrate was consumed by denitrifying bacteria. The data for DEA also showed denitrification occurred in all columns, regardless of treatment, at the surface where nitrification/denitrification coupling would occur. The NH_4^+ added to the columns underwent nitrification in oxidized zones within the soil (near the surface and within the rhizosphere) and as NO_3^- was formed by this process it diffused to anaerobic zones where it then underwent denitrification (Reddy and Patrick, 1984; Reddy and DeLaune, 2008).

Previous field systems showed influent nitrogen was being pumped into the marsh subsurface with nearly 100% of the wastewater nitrogen present as NH_4^+ (Turriciano, 2005; Fontenot et al., 2006). Laboratory experiments associated with this study have shown conversion of approximately 20% of the NH_4^+ to NO_3^- (via nitrification) before injection into thesubsurface (Appendix A). Data from this study suggests addition of NO_3^- to the marsh subsurface will result in reduced levels of nitrogen in the subsurface, as nearly 99% of NO_3^- is removed, most likely via denitrification (Chapter 5). If nitrification can be achieved in the collection tank prior to injection, then NH_4^+ loading can be reduced and less nitrogen will remain in the subsurface. This will aid in increasing the longevity of the system with regards to nitrogen for the MUS.

4.3.4 Ammonium Treatment

Ammonium was significantly ($p < 0.001$) reduced from an influent mean (94 ± 8 and 75 ± 7 mg NH_4^+-N mg L^{-1}) to overall mean column effluent (53 ± 26 and 44 ± 21 mg NH_4^+-N mg L^{-1}) for the 100%NH_4^+ and 80%NH_4^+/20%NO_3^- treatments, respectively. Removal efficiencies for NH_4^+ averaged 61% after five days and dropped to an overall removal efficiency of 19% by the end of the study. Previous MUS field studies found a 98% removal efficiency from an intermediate saltwater marsh (Turriciano, 2005) and a 99% removal efficiency from a saltwater marsh (Fontenot et al., 2006). The column study presented in this chapter was comparable to removal efficiencies from other treatment wetlands. A surface flow wetland receiving primary and secondary effluent from municipal sources had a removal efficiency of 17% (Knowlton et al., 2002), while single-family constructed subsurface flow wetlands treating domestic wastewater in Ohio, showed removal efficiencies ranging from 27 – 98%, with a mean of 56% (Steer et al., 2002). Removal efficiencies from surface flow wetlands in cold regions (Canada) treating dairy wastewater ranged from 90 – 94% (Smith et al., 2006). A surface flow wetland treating industrial wastewater showed a removal efficiency of 56% (Chen et al., 2006). A laboratory constructed wetland, which treated synthetic wastewater, achieved a removal efficiency of 82% over the course of 20 months (Wiessner et al., 2005). Generally, the final NH_4^+ removal efficiency (19%) from this study was lower than most other studies. However, it should be noted that in this study treatment was confined to soil contained within each experimental column. When the MUS is used in a field situation, the treatment area is not confined and the removal efficiency would be expected to be higher, in the short-term, as indicated by previous field studies. Long-term (> two years) effectiveness of the MUS for the treatment of nitrogen is not known.

Though the system was set up to represent plug flow treatment, NH_4^+ data from each depth was lumped and analyzed as a whole because of high variability in the data. The variability is believed to be due to temporary clogging caused by microbial growth which in turn likely caused frequents shifts in wastewater flow and changing channelization patterns in some regions of the column. The salinity*plants*nitrogen*time interaction was significant ($p < 0.05$), largely due to significant changes in NH_4^+ concentrations by time as wastewater was introduced into the columns (Figure 4.7). The overall means of the salinity, plants, and nitrogen effects also contributed to the significant salinity*plants*nitrogen*time interaction. For the nitrogen effect, the columns receiving 100 mg NH_4^+-N L^{-1} had a significantly higher NH_4^+ concentration than those columns receiving only 80 mg NH_4^+-N L^{-1}. For the salinity effect, the high salinity treatment was higher relative to the low salinity, while for the plants treatment, the planted treatment had a higher mean relative to the unplanted treatment.

The high salinity treatment had a higher mean effluent NH_4^+ concentration than the low salinity treatment. However, the low salinity treatment had a significantly higher ($p < 0.05$) concentration of exchangeable NH_4^+ (Figure 4.10) relative to the high salinity treatment. Higher salinities decrease the sorption of NH_4^+ due to higher concentrations of competing cations (Seitzinger et al., 1991), thus explaining the higher effluent NH_4^+ concentrations for the high salinity treatment. The low salinity columns are sorbing more NH_4^+, as expected, thus explaining the lower effluent NH_4^+ concentration. The increased availability of NH_4^+ at the low salinity also accounts for the higher exchangeable NH_4^+ as there was less competition from other cations. This implies for MUS installed in marshes of a higher salinity, saturation would occur sooner than for a MUS installed in a marsh of a lower salinity.

Planted columns had a significantly lower effluent NH_4^+ mean than unplanted columns. The surface (0 cm) depth*plants interaction term was also examined to determine any potential

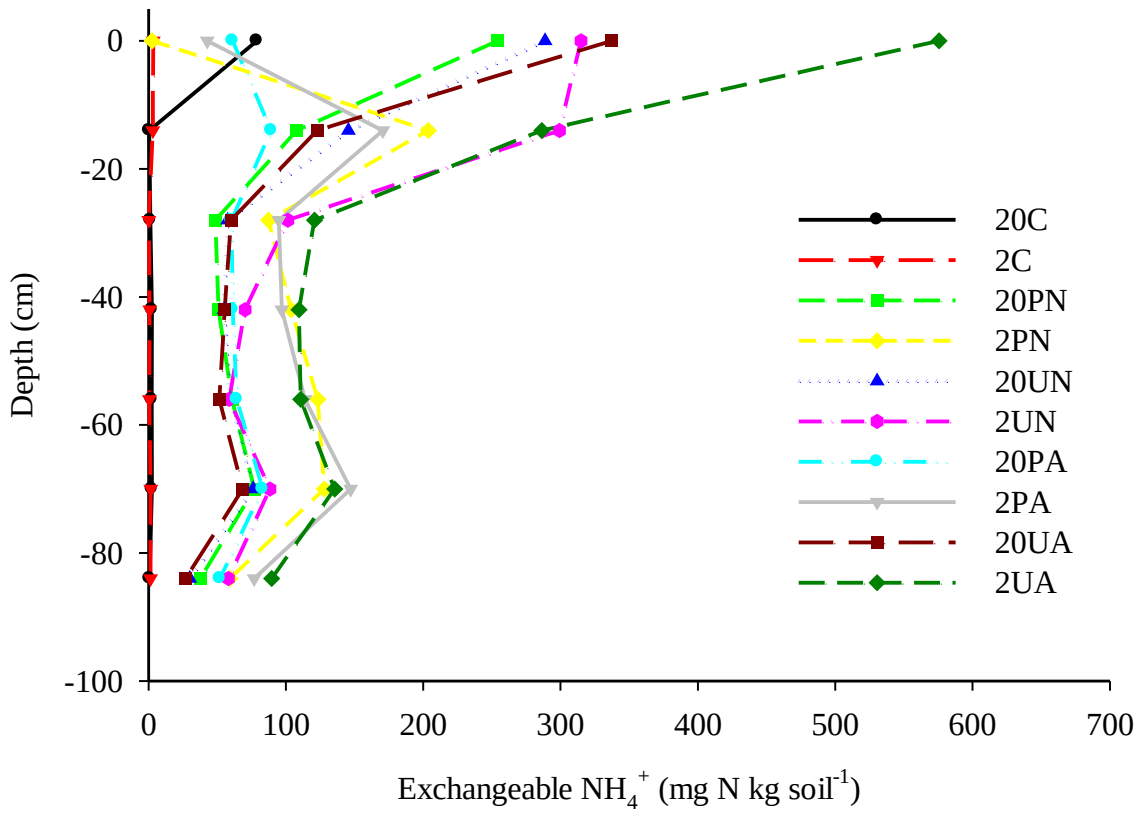


Figure 4.10 Mean exchangeable NH_4^+ by depth for individual treatment combinations. Treatments are abbreviated as planted (P) vs. unplanted (U), 100 mg NH_4^+-N L^{-1} (A) vs. 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N), and control (planted columns receiving no wastewater, C).

treatment by plants. The depth*plants at 0 cm was significant ($p < 0.05$), with the planted treatment being higher than the unplanted treatment. This indicated the difference between planted and unplanted treatments was potentially due to plant uptake. The significant finding of the plant*depth interaction was also supported by the significant differences ($p < 0.05$) seen in removal efficiency between planted (46%) and unplanted (38%) columns. Though the majority of the plants died partway through the study, there were two plants within wastewater treated columns that remained alive. Also, before plant death among the other columns, there was likely some uptake of NH_4^+. Plant uptake of NH_4^+ was further supported by the exchangeable NH_4^+ (Figure 4.10) data, as unplanted columns were significantly higher ($p < 0.05$) than planted, thus less NH_4^+ was taken up in unplanted columns. It is also possible that an oxidized rhizosphere

could lead to an increase in nitrification and, therefore, could also be an explanation for a decrease in NH_4^+. Though plants could provide significant treatment of NH_4^+, it is not desirable for untreated wastewater to reach the surface as this could indicate system failure.

Ammonium initially showed a high removal rate, but the removal rate began to taper off throughout the course of the study (Figure 4.11), showing breaks at day 14 and 56. After day 56, the removal rate began to slow, coming close to steady state. The initial high removal of NH_4^+ is most likely due to sorption of NH_4^+ to sorption sites within the soil. As these sites become saturated, less NH_4^+ can be sorbed and was then available for plant uptake, nitrification, or release into effluent. There were no significant differences of the removal rate between treatments.

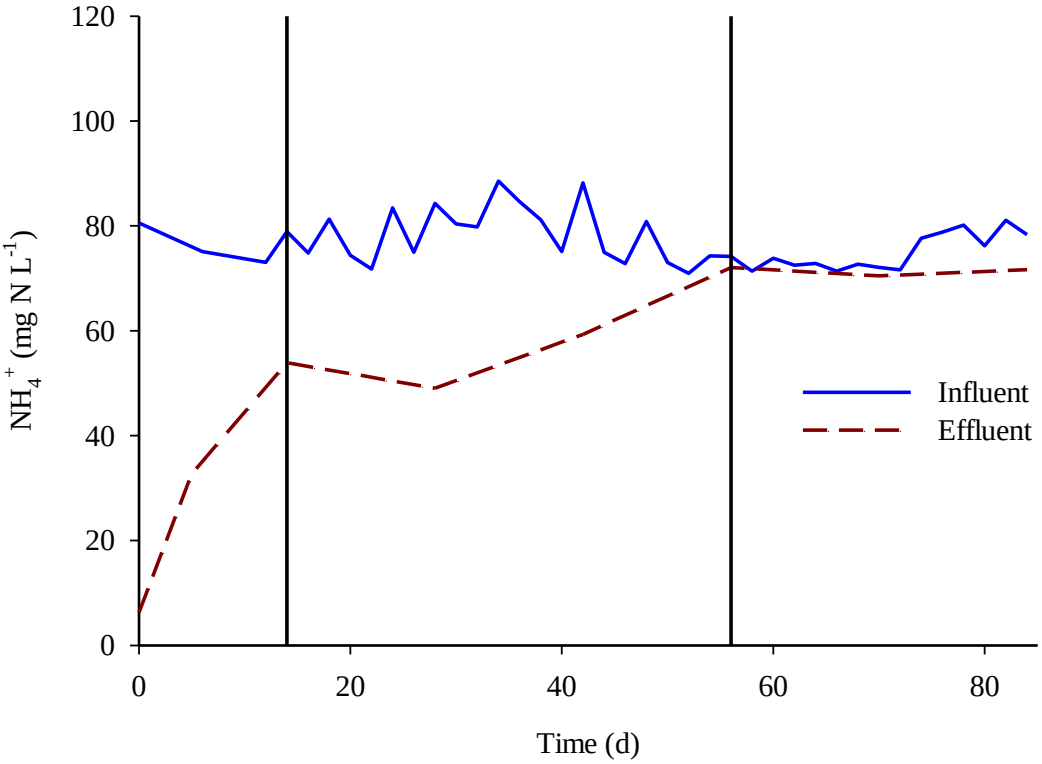


Figure 4.11 Representative graph of NH_4^+ versus time. The mean influent and mean surface effluent are presented. Vertical lines show days 14 and 56. Graph shows 20‰, unplanted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} treatment combination. Remaining graphs are presented in Appendix B.

Sorption is likely the primary method of NH_4^+ removal within the subsurface. There is some indication in this study that nitrification and plant uptake are important near the surface. However, the majority of NH_4^+ contained in the influent will be removed via sorption in the subsurface (for some unknown time period) before ever reaching the surface. The type of soil present in the wastewater plume will be important for the sorption of NH_4^+. Soils containing higher amounts of clay and organic matter will sorb more NH_4^+ than soils containing more sand, as sand is mostly inert and contributes little to sorption processes (Pierzynski et al., 2005). There are two types of NH_4^+ sorption to clay minerals: an ion exchange reaction that occurs on the surface of negatively charged clays (or exchangeable NH_4^+) and sorption into the interlayers of the clay structure for some clays (or fixed NH_4^+). Ammonium also sorbs to OM due to the natural negative charge organic matter carries. Generally the higher the OM content, the higher the NH_4^+ sorption (Rosenfeld, 1979; Hou et al., 2003; Fernando et al., 2005). Wastewater injection would increase the OM content over time and, subsequently, increase the CEC of the soil and, thus, increase its capacity to retain NH_4^+. Sorption by ion exchange to clay minerals and OM both fall into the cation exchange process where total NH_4^+ sorption is proportional to CEC (Dalal, 1975). In this study, the CEC was 14.5 centimoles of cation charge kg $soil^{-1}$ (or 2.03 g NH_4^+-N kg $soil^{-1}$), which was more than adequate to sorb the total amount of NH_4^+ loaded (0.54 and 0.43 g NH_4^+-N kg $soil^{-1}$ for the 100 and 80% NH_4^+-N treatments, respectively). Though the CEC of this soil was high enough to sorb the total amount of NH_4^+ loaded in this study, the data indicates the total amount of NH_4^+ loaded was not actually sorbed by the soil. Competition with other cations and some desorption probably played a role in the lack of NH_4^+ retention.

Salinity also has a profound effect on NH_4^+ sorption, which decreases with increased salinity (Gardner et al., 1991; Seitzinger et al., 1991; Rysgaard et al., 1999; Hou et al., 2003).

There is also an increased NH_4^+ desorption with increasing salinity (Rysgaard et al., 1999). This is partially due to increased competition with other cations as salinity is increased. Anions present in seawater also partially neutralize the charge of NH_4^+ ions, as anions form ion pairs with NH_4^+, and thus reduce the sorption of NH_4^+ to sediment particles (Dalal, 1975; Gardner et al., 1991; Rysgaard et al., 1999). In field systems, the type of soil, quantity of OM, and groundwater salinity will all affect the sorption of NH_4^+. Consideration of these characteristics is important to the long-term sustainability of the MUS. The treatment area will also play a large role in the longevity of the system, as larger volumes will lengthen the time frame in which the MUS can operate effectively. Additionally, consideration of increased NH_4^+ loads due to overlapping plumes should be considered if the MUS is to be installed at adjacent camps, as this would lead to a decrease in the longevity of the system.

4.4 Conclusion

This study found DOC to be treated with a removal efficiency of 90%. A higher salinity had a significant negative impact on the treatment of DOC. However, as time progressed the treatment of DOC under the high salinity columns improved, suggesting the microbial population needs a longer acclimation period at the high salinity for the treatment of carbon. Nitrate (as NO_X) was removed with > 99% efficiency. Thus, prior conversion of NH_4^+ to NO_3^- in the collection/distribution tank before injection into the marsh subsurface would significantly improve the overall treatment of nitrogen within the MUS, as NO_3^- would undergo denitrification after injection into the subsurface and, subsequently, be removed from the system as N_2 gas diffuses into the atmosphere. Salinity had no significant impact on NO_X treatment. Though NH_4^+ removal efficiencies eventually fell to levels near 20%, an increased treatment area and higher amounts of clay and organic matter (found within field systems) would increase NH_4^+ treatment effectiveness. However, consideration of overlapping wastewater plumes should be

considered in relation to sorption treatment of NH_4^+ if the MUS is installed at adjacent camps, as it would decrease the treatment area available. Salinity had a significant negative impact on the treatment of NH_4^+ with increased salinity, as more NH_4^+ was released into the effluent at the high salinity. Even though the majority of the plants died during the course of the study, plants still had a significant impact on the treatment of both NH_4^+ and NO_X. Plants decreased the NH_4^+ level within the soil, presumably through uptake and introduction of oxygen through the rhizosphere, which increased nitrification (and subsequently increased NO_X levels). Nitrification/denitrification coupling would then ultimately convert more NH_4^+ to N_2, which would be removed from the system. Consideration of nitrification/denitrification coupling combined with the knowledge of high salinity and plant impacts on nitrogen treatment will aid in increasing the treatment longevity and long-term sustainability of the MUS for treatment of wastewater nitrogen in coastal wetland systems.

CHAPTER 5: SOIL AND MICROBIAL PROPERTIES RELATING TO THE TREATMENT OF NITROGEN WITHIN THE MARSHLAND UPWELLING SYSTEM

5.1 Introduction

Nitrogen cycling is globally important as nitrogen is essential for living tissues, comprising an integral part of enzymes, which mediate carbon reducing or oxidizing biogeochemical reactions. Nitrogen also plays a role in the degradation of water quality due to eutrophication. The rate of primary productivity, in a variety of ecosystems, is often limited by nitrogen. (Schlesinger, 1997). It has been estimated that the amount of plant available nitrogen would become too low to be sustainable within a week if it were not for the action of microorganisms involved in the nitrogen cycle (Maloy and Schaechter, 2006). Microorganisms serve as a driving force in the global nitrogen cycle and are key to the transformation of organic and inorganic nitrogen pools, such as the transformations brought about by mineralization, nitrification, and denitrification.

Nitrogen mineralization is the breakdown of organic nitrogen to inorganic forms. The primary pathway for mineralization of organic nitrogen in wetlands is ammonification. This process, which is performed by a wide variety of heterotrophic microorganisms, converts organic nitrogen to NH_4^+. Mineralization occurs under both aerobic and anaerobic conditions. The complex organic nitrogen compounds present in the soil are hydrolyzed by extracellular enzymes into simple monomers (Gardner et al., 1989; McLatchey and Reddy, 1998; DeBusk et al., 2001). Enzymes eventually break down the organic nitrogen into simpler compounds, followed by a breakdown of the amino acids that subsequently results in the liberation of NH_4^+ deaminase (Gardner et al., 1989; DeBusk et al., 2001).

Nitrifiers convert NH_4^+ to NO_3^- in aerobic environments (i.e., the water column, upland soils, aerobic soil layer, and aerobic portions of the rhizosphere). These autotrophic bacteria

couple the oxidation of NH_4^+ to electron transport phosphorylation while utilizing inorganic carbon to synthesize cellular components required for microbial growth (DeBusk et al., 2001). The availability of oxygen, NO_2^-, alkalinity, and NH_4^+ are the primary limiting factors of nitrification (Metcalf and Eddy, 2003). In the majority of wetlands, NH_4^+ is supplied to aerobic layers through transport (via diffusion) from anaerobic/anoxic soil layers due to a concentration gradient across the two layers. While plants increase the area in which nitrification can occur in wetlands by virtue of their rhizosphere, they can also serve as competitors through uptake of NH_4^+ as a nutrient source.

In juxtaposition to nitrifiers, denitrifiers convert NO_3^- to N_2O or N_2 under anaerobic conditions and remove the majority of nitrogen in wetlands. Denitrifiers are facultatively anaerobic, although denitrification can only proceed in an anoxic environment (Schlesinger, 1997). Denitrifiers will preferentially use oxygen, if present, as their terminal electron acceptor; therefore, the redox status of wetland soils can control the extent to which denitrification is able to occur within the soil profile (Yu and Patrick, 2004). As a result, denitrification is not likely to occur in a soil until all oxygen has been depleted. Nitrate is often provided by diffusion from the overlying water column, the thin oxygenated surficial soil, or the rhizosphere due to the process of nitrification. Plants may also serve as competitors for NO_3^- as a nutrient source, even though plants can enhance denitrification as a source of NO_3^- through their rhizosphere (via nitrification). Wetland soils commonly have conditions that are more conducive to denitrification than upland soils, such as a higher organic carbon content and lower oxygen status. These conditions are due to poor drainage and presence of floodwaters that are indicative of wetlands that restrict oxygen resupply (DeBusk et al., 2001). Denitrification is the primary process of inorganic nitrogen removal from wetland ecosystems and, therefore, wetlands often serve to significantly enhance water quality (DeBusk et al., 2001).

Wetlands receive nitrogen inputs from adjacent upland areas, retain nitrogen, and, because of their location in the ecosystem, often act as net organic nitrogen sinks. Understanding the role of microbially-mediated processes within wetlands has become important as microbes are vital to the fate of nitrogen. Soil microbes can exert a significant influence on the energy flow of a wetland ecosystem because mineralization of organically bound nutrients regulates nutrient availability for both primary production and decomposition (Elliott et al., 1984). Thus, microbial communities are vital for transforming nitrogen within wetland ecosystems. A novel wastewater treatment system being tested in coastal wetland environments is the marshland upwelling system (MUS), which capitalizes, in part, on the nitrogen processing of microbes. The MUS was developed as an alternative wastewater treatment system to address the special issues that are present the coastal environments of Louisiana, such as high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater (Stremlau, 1994; Watson Jr. and Rusch, 2001, 2002; Richardson et al., 2004; Richardson and Rusch, 2005; Turriciano, 2005; Fontenot et al., 2006; Evans and Rusch, 2007b, a). The majority of current onsite wastewater treatment and disposal systems technologies, such as septic systems, do not work well under the intermittently or constantly flooded conditions found in wetland environments. The MUS (Figure 5.1) utilizes the natural properties of the soil beneath the prevalent natural wetlands in coastal areas. An effectively operating system is dependent upon the native groundwater salinity, the injection frequency and flow rate, and the natural filtering properties and microbial transformation processes of the native soil matrix. The system consists of a collection/distribution tank, injection pump, programmable timer, injection well, and saturated subsurface soils.

Previous field studies have looked at the short-term effectiveness of the MUS on wastewater treatment in both salt and intermediate marshes (Watson Jr. and Rusch, 2002;

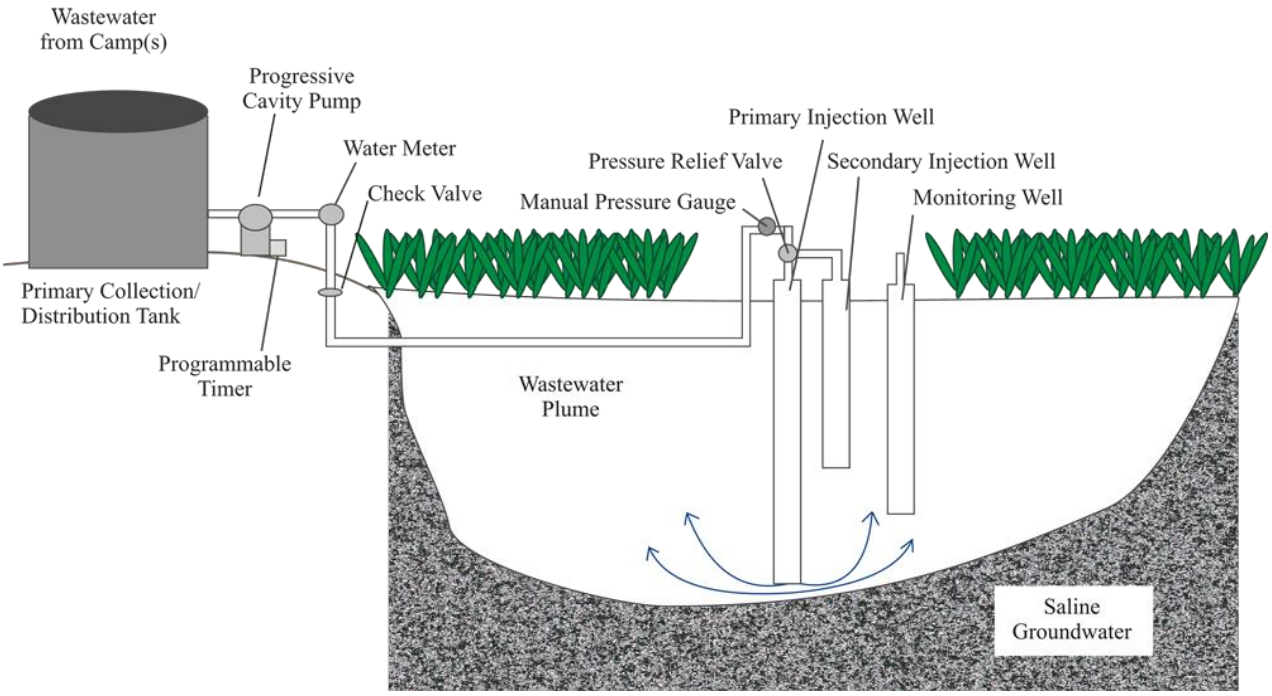


Figure 5.1 Generic schematic of the marshland upwelling system.

Richardson and Rusch, 2005; Turriciano, 2005; Addo et al., 2006; Fontenot et al., 2006; Evans and Rusch, 2007a). Various researchers have found that with an increase in salinity there is a decrease in microbially-mediated nitrogen activity (Gardner et al., 1991; Seitzinger et al., 1991; Stehr et al., 1995; Rysgaard et al., 1999; de Franca et al., 2000; Yoshie et al., 2001; Bollmann and Laanbroek, 2002; Rietz and Haynes, 2003; Yoshie et al., 2004; Grommen et al., 2005; Seo et al., 2008). Because the MUS is being developed for regions of varying salinity, understanding the effects of salinity on nitrogen treatment within the MUS is important for system design under different salinities. The MUS has demonstrated effective short-term treatment for nitrogen in field studies. However, the sustainable longevity of the system for nitrogen treatment is uncertain. Subsequently, a better understanding of the specific processes occurring could potentially improve the system for long-term usage.

Thus, the objectives for this study were to: 1) determine what nitrogen transformations were occurring within the soil, 2) determine the fate of nitrogen within the MUS, and 3) determine potential influences (including salinity) on microbially-mediated processes.

5.2 Materials and Methods

5.2.1 Experimental Setup

Nitrogen transformations within the MUS were investigated using a laboratory column experiment because field conditions are essentially unbounded, thus preventing a quantitative and detailed process investigation. The removal of nitrogen was evaluated under varying conditions chosen to simulate field conditions as closely as possible. Three experimental treatments (salinity, nitrogen, and plants), with two levels each were chosen to evaluate the efficiency of nitrogen removal (Figure 5.2). The nitrogen levels were chosen as representative of levels seen at field sites (Turriciano, 2005; Fontenot et al., 2006). To determine the amount of NO_3^- to use, a small laboratory study looked at the potential maximum amount of conversion from NH_4^+ to NO_3^- achievable if wastewater was aerated before injection into the subsurface (Appendix A). Each salinity treatment also had a planted control that received no wastewater to compare wastewater treated columns to control columns receiving only the corresponding saltwater. Treatment combinations were duplicated and randomly placed for a total of 16 wastewater-treated columns and 4 control columns.

Columns were made from 15.24 cm (inner diameter) PVC pipe. Four columns were made from clear PVC pipe to allow for viewing of soil, but were covered with aluminum foil to prevent light from entering the subsurface and contributing to algal or microbial growth. The bottom of each column (Figure 5.3) was fitted with a distribution plate to allow for even flow of the incoming wastewater as it passed through the gravel and sand layers. This ensured the entire bottom surface of the soil received the wastewater homogenously. Sampling ports were installed at 0, 8, 34, 58, and 84 cm below the surface of the soil. On the opposite side of the column from sampling ports, septa were installed to allow for measurement of redox potential. Platinum wire pieces of 2.54 cm were inserted through septa to permit redox potential readings to take place

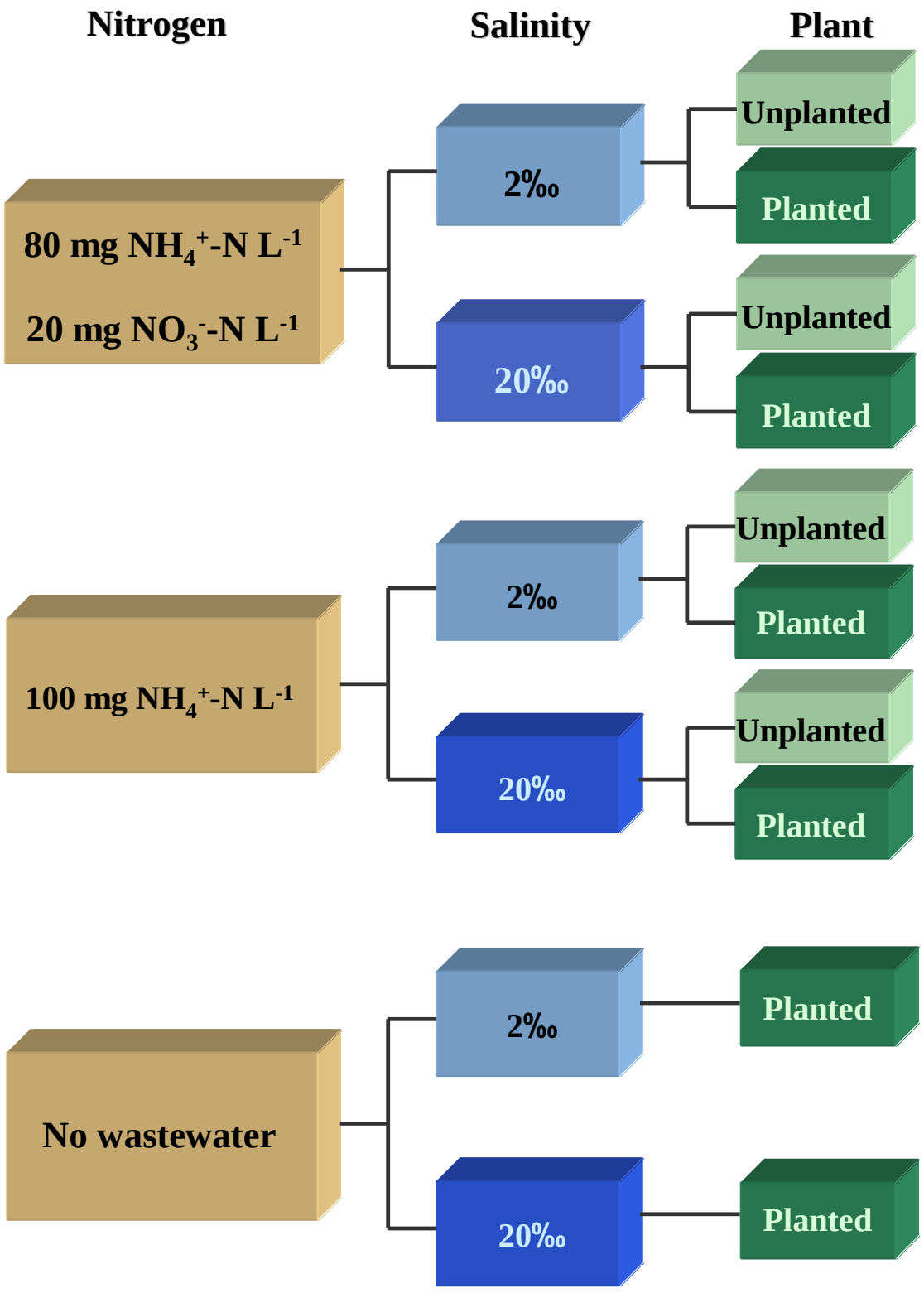
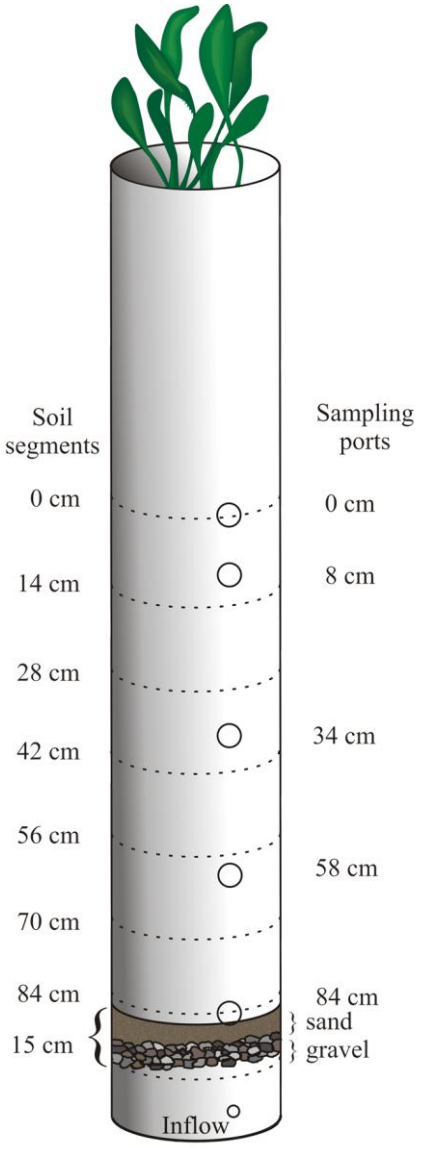


Figure 5.2 Layout of experimental design showing three treatments used. Each treatment combination was duplicated for a total of 20 experimental units (or columns). Parts per thousand is abbreviated as ‰.

within the subsurface. The bottoms of the columns were filled with 7.6 cm of gravel and 7.6 cm of sand to prevent the distribution plate from clogging with the clay and silt present, as the holes in the distribution plate were approximately 0.16 cm in diameter.

Soil collected from a salt marsh in Port Fourchon, Louisiana was homogenized, sterilized, and placed in each column up to a depth of 69 cm. In order to ensure measured microbial activity was due to microbes native to a particular salinity, soil was sterilized. Soil sterilization prevented introduction of saltmarsh microorganisms into the columns under the low salinity treatment, as soil was originally obtained from a salt marsh. Additional soil containing a higher amount of organic matter was collected from a saltmarsh in Port Fourchon, Louisiana, and an intermediate marsh near Westwego, Louisiana, and was homogenized before adding to the columns to bring the total soil height to 84 cm. This organic matter was added to columns of the corresponding salinity. The addition of the organic matter to the surface was done in order to achieve a soil profile more closely resembling the soil profile seen at field sites.



Saltwater of the target salinity was flushed through the columns until they reached the target salinity for their respective treatments. The columns were then inoculated with microorganisms from the appropriate marsh salinity.

Figure 5.3 Schematic of column setup. Sampling port depths are indicated on right. Soil segments cut from column at end of experiment are indicated on left. The distribution plate was installed beneath gravel.

Microorganisms were extracted from the soil following the procedure by Riis et al., (1998). The procedure was only followed to step one as subsequent steps were for the isolation of microorganisms, which was not desired in this experiment. After centrifugation, the microorganisms were resuspended in 2.3 L of a 0.2% $Na_2P_2O_7$ solution and injected into the bottom four sampling ports. Columns receiving the planted treatment were planted with *Paspalum vaginatum* and *Spartina alterniflora* according to their native salinities, 2 and 20‰, respectively. An inert plastic mesh (~ 0.32 cm) was placed at the interface (15 cm below the surface) between the subsurface soil and higher organic matter surface soil to prevent roots from growing below that depth. Light banks were installed and maintained at approximately 200 μmol s^{-1} m^{-2} for approximately 12 hours each day to ensure plant growth was not inhibited by lack of light.

Standard synthetic wastewater (ASTM, 2006) was used with the following amendments: salt, nitrogen, and phosphorus levels were increased to the desired wastewater concentrations, kaolin (a mineral) was not added, a supplement (Reef Plus, Aquatic Ecosystems) containing trace nutrients was added, and the amount of beer (carbon source) was decreased from 6% to 1%. The amount of beer was decreased because initial tests found the dissolved organic carbon (DOC) level to be too high in comparison to field wastewater levels. Wastewater-treated columns received 12 mg PO_4^{3-}-P L^{-1} and 200 mg C L^{-1}. Saltwater solutions were made up using individual components (Table 5.1) (Atkinson and Bingman, 1998) as opposed to a commercial ocean salt mix because initial tests found the sulfide levels (from reduction of seawater sulfate) to be extremely high in some columns (22 mM S^{2-} in one column), to the point of toxicity to plant growth. Sulfate is reduced to sulfide under strongly reduced conditions, such as occurred in this study. Koch and Mendelssohn (1989) found that just 1 mM S^{2-} significantly decreased the growth of two marsh plants. Field studies of the MUS have shown very little sulfide exists

Table 5.1 Concentrations of salts used in synthetic wastewater for the 2 and 20‰ and 100 mg NH_4^+-N L^{-1} (A) and 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N) treatments.

	Treatment			
	2A	2N	20A	20N
Salts	Concentration (g L^{-1})			
NH_4Cl	0.38	0.30	0.38	0.30
KNO_3	--	0.15	--	0.15
$Na_3PO_4 \cdot 12H_2O$	0.18	0.18	0.18	0.18
$NaHCO_3$	0.25	0.25	0.25	0.25
$MgCl_2 \cdot 6H_2O$	0.62	0.62	6.25	6.25
$CaCl_2 \cdot 2H_2O$	0.09	0.09	0.85	0.85
KCl	0.04	--	0.29	0.29
NaCl	0.62	0.20	16.00	15.80
K_2SO_4	0.002	0.002	0.02	0.02

within the MUS wastewater plume, justifying the reduction of sulfur levels to 0.1 (20‰) and 0.01 (2‰) mg SO_4^{2-}-S L^{-1}.

Two L of synthetic wastewater were pumped into the bottom of each column every other day, equivalent to an injection flow rate of 0.03 L min^{-1}. The addition of wastewater took approximately an hour, which was equal to a mean flowrate of 0.7 mL min^{-1} over two days and led to a calculated complete turnover of wastewater within the column porewater approximately every eight days. Wastewater was sampled at every addition which was every two days. Samples were collected from each sampling port (before wastewater addition) on days 0, 5, 14, and every 14 days thereafter for a total of 84 days. Salinity, pH, and temperature of the samples were measured at time of collection. Redox potential of the soil was also measured on the collection day using a SCE reference electrode pushed into the surface of the columns along with the platinum wire inserted through the septa. Surface redox potential was measured using a platinum electrode inserted into the soil to a depth of 5 cm. All redox measurements (E_c) were corrected to a standard hydrogen reference electrode and expressed as E_h. At the end of the

study, columns were cut into seven sections as shown in Figure 5.3. Each section was then mixed to ensure a homogenous mixture of sediment and stored at 4°C until further analysis.

5.2.2 Soil and Microbial Analyses

Sand, silt, and clay fractions were measured using sieve (ASTM C117, C136) and hydrometer analyses (ASTM D422) (ASTM, 1995). X-ray diffraction analysis was performed in the Louisiana State University Department of Geology X-ray crystallography laboratory using a Bruker/Siemens D5000 automated powder X-ray diffractometer (Madison, WI) with Rietveld analysis software. Pre-study, soils were measured for organic matter (OM) content, percent moisture, and total carbon and nitrogen (TC and TN). Post-study, soils were measured for bulk density, organic matter (OM) content, percent moisture, and total carbon and nitrogen (TC and TN). Bulk density was calculated for each soil segment, excluding the sand/gravel segment, on a dry weight basis. Organic matter was measured as loss on ignition at 435°C (Sparks, 1996). Percent moisture was calculated (as g of water in soil sample * 100/g dry soil in sample) on ~ 10 g soil dried at 70°C until constant weight (Klute, 1986). Total C and TN were determined on dried, ground samples using a Costech Elemental Combustion System (Valencia, CA).

Soil sections were analyzed for microbial biomass carbon (MBC), denitrification enzyme activity (DEA), exchangeable NH_4^+, and potentially mineralizable nitrogen (PMN). Extractants from MBC, exchangeable NH_4^+, and PMN were filtered using 0.45 µm membrane filters and stored at 4°C until analysis. Microbial biomass C was measured using ~ 2 g moist soil following the chloroform fumigation method after Vance et al., (1987) and White and Reddy (2001) and measured directly using a Shimadzu TOC-V_{CSN} analyzer (Columbia, MD). Denitrification enzyme activity was measured using ~ 5 g moist soil in glass serum bottles evacuated with 99.99% O_2-free N_2 gas after White and Reddy (1999). The N_2O for the DEA analysis was measured using a Shimadzu GC-8A equipped with an ECD detector (Columbia, MD).

Exchangeable NH_4^+ was determined following Mulvaney (1996) with the following amendments: 25 mls of KCl was added to ~ 2 g moist soil in a 30-ml centrifuge tube and shaken for 30 min. The PMN rates were determined by adding 10 mL of distilled, deionized water to ~ 5 g of moist soil in glass serum bottles evacuated with 99.99% O_2-free N_2 gas (White and Reddy, 2001). Samples for PMN analysis were incubated for 10 days at 40°C, extracted with 2M KCl and compared to exchangeable NH_4^+ samples (time-zero controls). Ammonium for exchangeable NH_4^+ and PMN was preserved at pH < 2 and measured using a Seal Analytical AQ2 Automated Discrete Analyzer (Mequon, WI) following EPA method 350.1 (USEPA, 1993).

5.2.3 Statistical Analyses

SAS® software (2009) and SigmaPlot® software (2002) were used to analyze the data. All statistical tests were performed at a significance level of $\alpha = 0.05$. A three-way ANOVA (between salinity, plants, and nitrogen) with a split-plot addition for depth was applied to compare treatments. Controls were excluded from the three-way ANOVA as they led to an unbalanced design. In order to compare controls to wastewater treated columns, comparisons to control data were made using a one-way ANOVA (using the 10 treatment combinations) with linear combinations between controls and wastewater treated columns. Soil characteristics were related using Pearson's correlation analysis.

5.3 Results and Discussion

5.3.1 Soil Characteristics

The soil added to the columns was found to be a silt loam from 0 – 15 cm and a loamy sand from 15 – 84 cm (Table 5.2). At the end of the study, the columns were sectioned for a total of 120 soil samples and 20 sand/gravel samples from the bottom of each column. Analysis of soil and microbial data found, for all parameters measured (excluding bulk density), the mean

Table 5.2 Percent sand, silt, and clay and initial mean organic matter content, total carbon, and total nitrogen for soil used in columns.

	cm below surface		
	0 - 15		15 - 84
Property	20‰	2‰	all
sand (%)	13	22	78
silt (%)	72	58	18
clay (%)	15	20	4
USDA classification	silt loam	silt loam	loamy sand
organic matter (%)	24	77	1.3
total carbon (g C kg soil^{-1})	-	-	10
total nitrogen (g N kg soil^{-1})	-	-	BDL[a]

[a]Below detection limit of 0.5 g N kg-soil^{-1}.

was higher at the surface and decreased with depth (Table 5.3, Figures 5.4 – 5.6). Bulk density showed the opposite trend and increased with depth (Table 5.3). There were no significant differences seen in the soil sections from 28 – 84 cm (below the surface) and the sand section. As such, this data is averaged together for graphical and tabular presentation in order to make the data more clear. The top 15 cm of soil in each column contained OM obtained from a marsh of a similar salinity in order to better simulate field sites, thus from 0 – 14 cm the soil was entirely comprised of high OM material and from 14 – 28 cm the soil was a mixture of the surface OM layer and the soil found from 28 – 84 cm. This accounted for the mid-range values found from 14 – 28 cm for most parameters (Table 5.3, Figures 5.4 – 5.6).

Organic matter content at the end of the study ranged from 1.6 – 62%, with the lowest percentages found in the subsurface (28 – 84 cm) (Table 5.3). Significance was found for the salinity*plants*nitrogen*depth interaction ($p < 0.05$). The significance in OM content was only found from 0 – 14 cm, where both low salinity, unplanted treatments had a significantly higher OM content relative to all other treatments at this depth (Figure 5.7). The initial percentages of OM account for the significant differences seen in OM. Initially, the OM content of the low salinity treatment was 77%, while the high salinity treatment was 24%. The initial difference in OM content was because of the initial setup where soil collected from marshes of similar

Table 5.3 Mean soil percent moisture, organic matter content, bulk density, and total carbon and nitrogen by depth for control and wastewater treated columns at the end of the study. The 28 – 84 cm depth includes the sand layer, except for bulk density, for which it was not measured.

		Parameter				
Treatment	Depth (cm)	moisture (%)	organic matter (%)	bulk density (g cm^{-3})	total carbon (g C $kg\ soil^{-1}$)	total nitrogen (g N $kg\ soil^{-1}$)
20PC	0 - 14	118 ± 7.7	10 ± 2.0	0.49 ± 0.11	31 ± 11	1.9 ± 0.6
	14 - 28	69 ± 21	4.7 ± 0.02	0.84 ± 0.11	15 ± 2.0	0.91 ± 0.21
	28 - 84	27 ± 3.2	2.2 ± 0.6	1.48 ± 0.04	6.3 ± 1.0	BDL[a]
2PC	0 - 14	186 ± 50	10 ± 0.7	0.18 ± 0.21	61 ± 13	4.0 ± 0.7
	14 - 28	78 ± 12	3.0 ± 0.9	0.72 ± 0.14	13 ± 1.3	0.84± 0.16
	28 - 84	28 ± 3.9	1.6 ± 0.7	1.47 ± 0.05	6.3 ± 0.9	BDL
20PN	0 - 14	195 ± 63	19 ± 4.3	0.28 ± 0.04	83 ± 27	5.2 ± 0.8
	14 - 28	58 ± 1.9	4.5 ± 0.6	0.95 ± 0.02	17 ± 1.1	1.2 ± 0.2
	28 - 84	26 ± 4.3	2.1 ± 0.6	1.44 ± 0.04	6.4 ± 2.0	BDL
2PN	0 - 14	156 ± 10	11 ± 0.9	0.39 ± 0.02	46 ± 4.7	5.1 ± 0.2
	14 - 28	85 ± 1.8	4.3 ± 3.3	0.67 ± 0.001	18 ± 16	0.97 ± 1.4
	28 - 84	26 ± 4.7	2.1 ± 0.7	1.50 ± 0.07	5.9 ± 1.7	BDL
20UN	0 - 14	246 ± 26	20 ± 1.9	0.20 ± 0.08	80 ± 0.7	5.8 ± 0.06
	14 - 28	77 ± 41	6.6 ± 3.8	0.86 ± 0.34	23 ± 13	1.6 ± 1.1
	28 - 84	26 ± 5.1	2.0 ± 1.0	1.45 ± 0.05	6.4 ± 2.0	BDL
2UN	0 - 14	833 ± 34	62 ± 2.7	0.07 ± 0.02	301 ± 5.6	22 ± 0.7
	14 - 28	108 ± 18	9.0 ± 0.1	0.57 ± 0.13	42 ± 12	3.1 ± 0.7
	28 - 84	26 ± 4.1	2.1 ± 0.9	1.47 ± 0.11	5.8 ± 1.6	BDL
20PA	0 - 14	104 ± 6.3	9.8 ± 2.0	0.52 ± 0.06	38 ± 2.7	2.4 ± 0.4
	14 - 28	55 ± 9.3	4.5 ± 1.1	0.96 ± 0.14	14 ± 4.6	0.89 ± 0.27
	28 - 84	26 ± 3.6	3.3 ± 1.2	1.45 ± 0.05	6.5 ± 0.9	BDL
2PA	0 - 14	141 ± 6.2	9.8 ± 0.2	0.38 ± 0.05	46.4 ± 9.9	3.4 ± 0.8
	14 - 28	78 ± 12	5.9 ± 0.3	0.77 ± 0.06	25 ± 5.1	1.8 ± 0.2
	28 - 84	26 ± 4.8	2.2 ± 0.9	1.44 ± 0.06	6.2 ± 1.6	BDL
20UA	0 - 14	222 ± 17	18 ± 2.7	0.22 ± 0.001	77 ± 13	5.6 ± 0.7
	14 - 28	73 ± 16	4.5 ± 1.7	0.82 ± 0.16	19 ± 0.1	1.1 ± 0.2
	28 - 84	24 ± 5.5	2.7 ± 1.0	1.47 ± 0.06	6.1 ± 2.0	BDL
2UA	0 - 14	564 ± 177	46 ± 15	0.10 ± 0.04	225 ± 87	17 ± 6.2
	14 - 28	87 ± 33	6.4 ± 1.9	0.72 ± 0.24	28 ± 7.8	2.0 ± 0.9
	28 - 84	26 ± 5.1	1.6 ± 1.0	1.44 ± 0.08	4.9 ± 2.6	BDL

[a]Below detection limit of 0.5 g N kg-$soil^{-1}$.

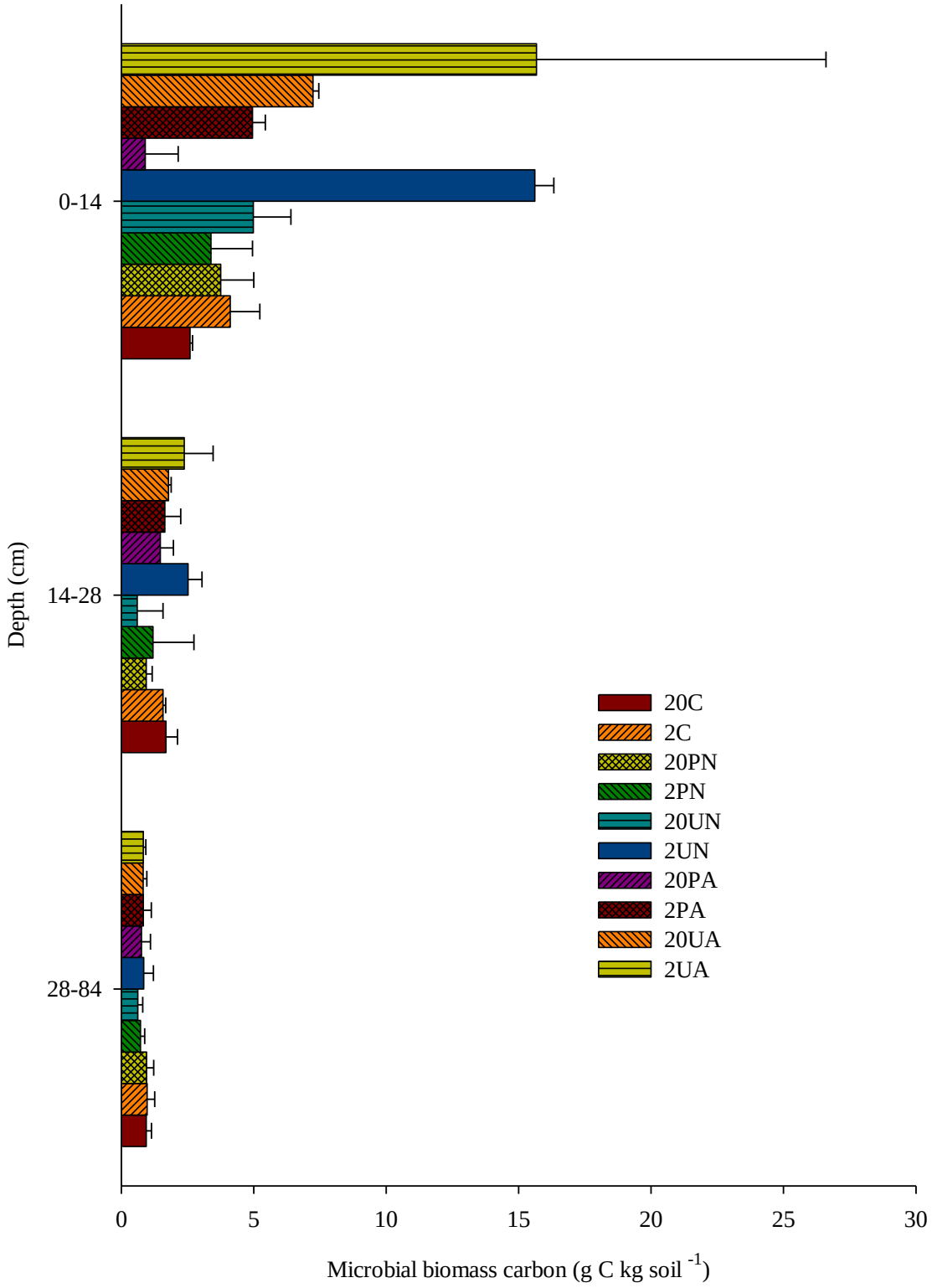


Figure 5.4 Mean microbial biomass carbon of columns by depth below surface. Error bars show variability between duplicates of each treatment combination. Treatments are abbreviated as follows for all figures: 2‰ (2), 20‰ (20), planted (P), unplanted (U), 100 mg NH_4^+-N L^{-1} (A), 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N), and control (planted columns receiving no wastewater, C).

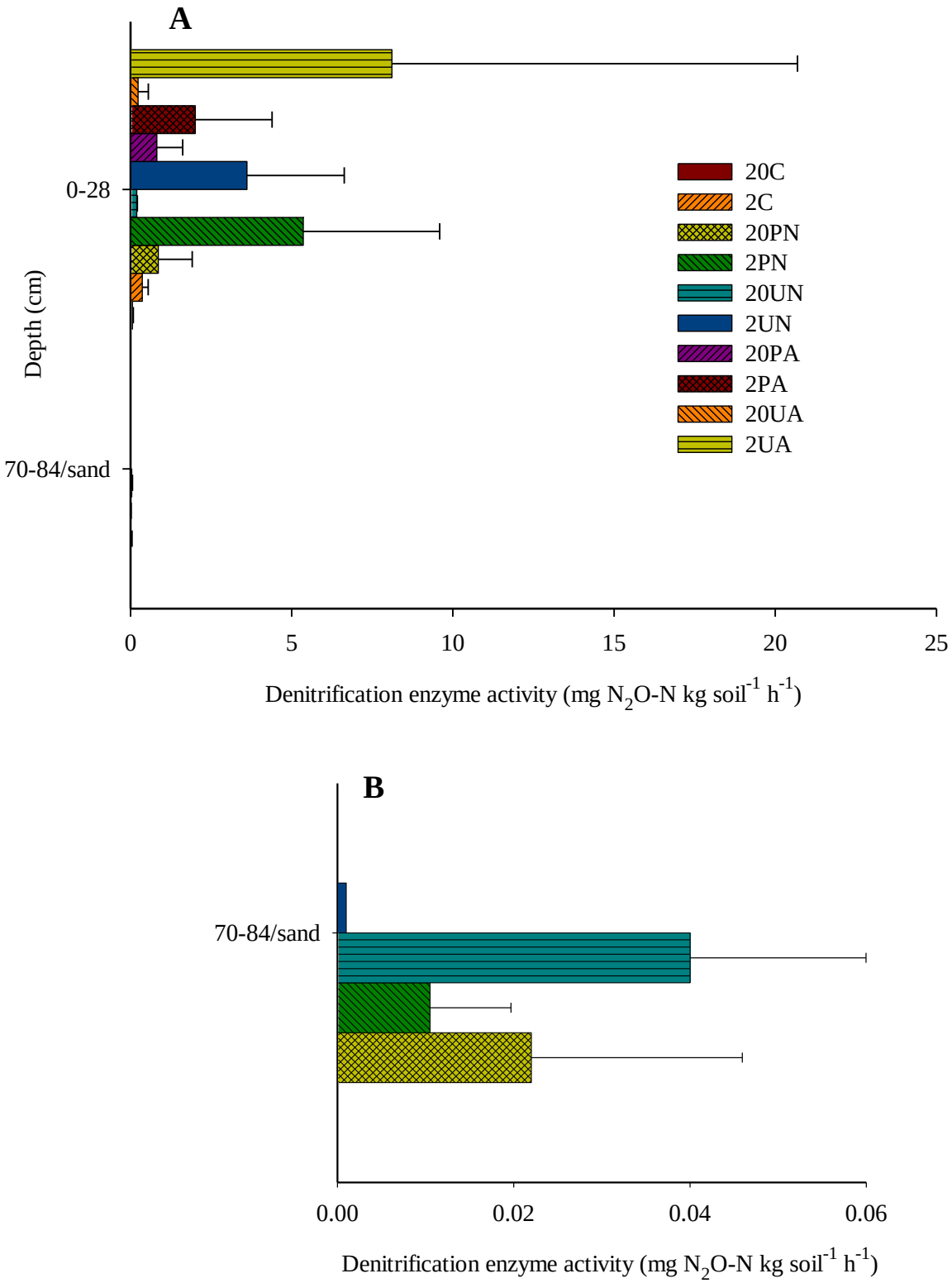


Figure 5.5 Mean denitrification enzyme activity (DEA) of columns by depth below surface. Data not shown is below detection limit (0.001 mg N_2O-N kg $soil^{-1}$ h^{-1}). Graph A shows DEA of all treatments. Graph B shows the 70 – 84 cm/sand section on a smaller scale. Error bars show variability between duplicates of each treatment combination.

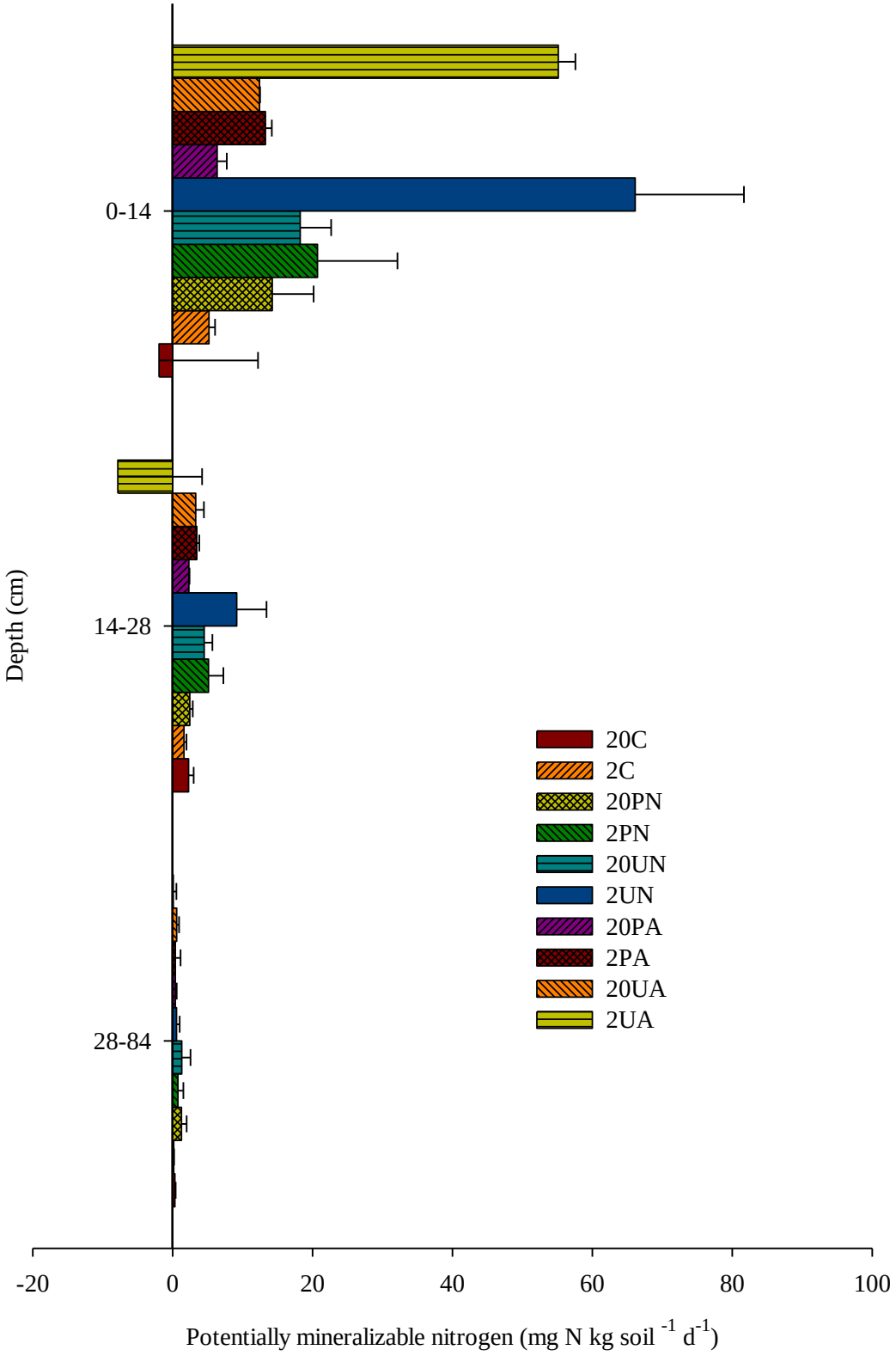


Figure 5.6 Mean potentially mineralizable nitrogen of columns by depth below surface. Error bars show variability between duplicates of each treatment combination.

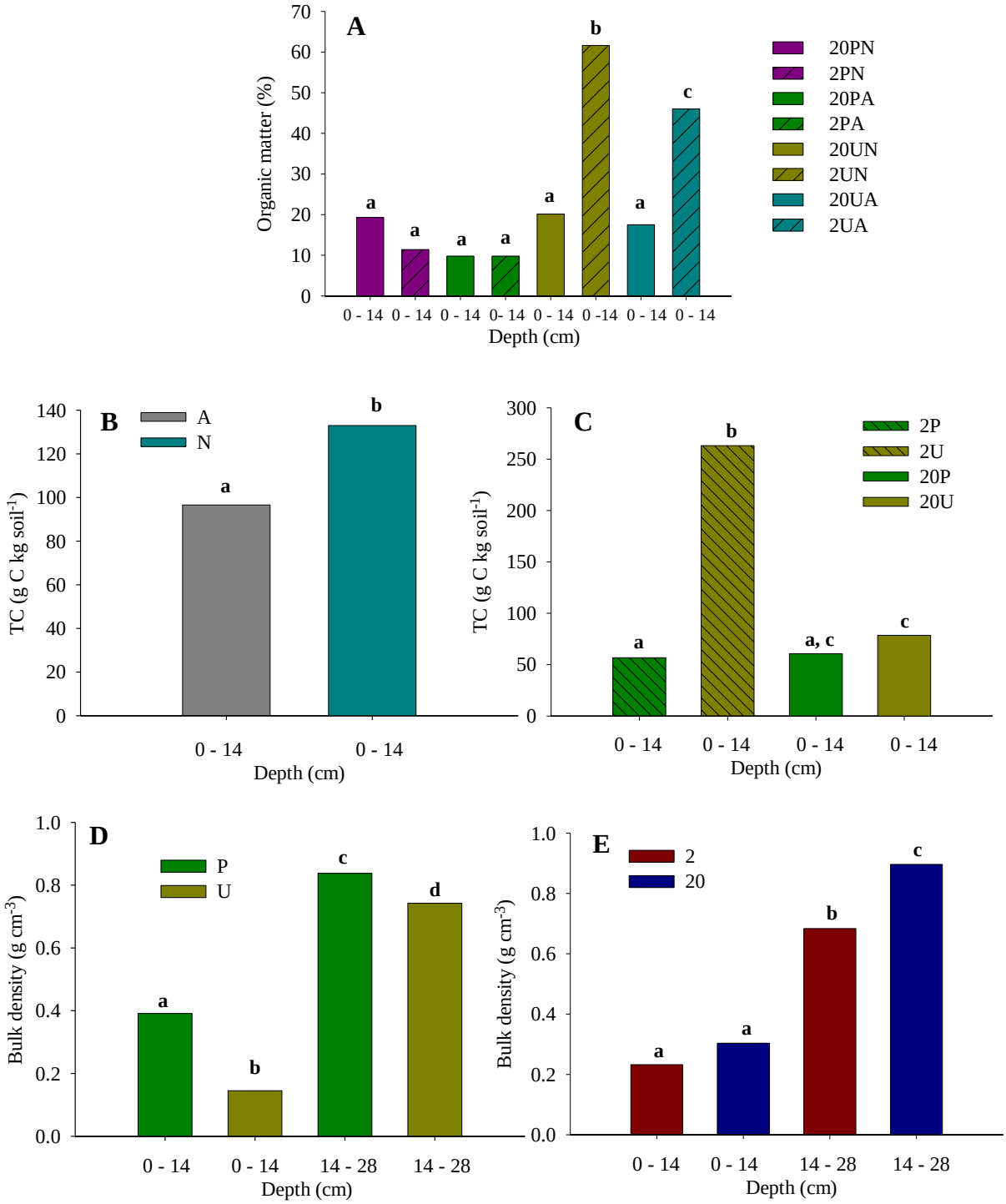


Figure 5.7 Significant statistical comparisons of soil characteristics. Letters indicate treatment combinations with significant differences. Treatments are not compared to controls as comparison to controls was done with a different analysis. Only significant depths are shown, all depths not shown were found to have no significant difference. Graph A shows organic matter content, B and C show total carbon (TC), and D and E show bulk density.

salinities was added to better simulate the OM content of natural wetlands in previous MUS field sites.

The surface OM content decreased from 77% to 32 (± 25%) and from 24% to 17 (± 5%) for the low and high salinity treatments, respectively, during the course of this study (Tables 5.2 and 5.3). This was likely due to microbial processes that consumed the readily available organic carbon present. In contrast to this decrease, the subsurface increased from 1.3% to a mean of 2.5 (± 0.9%). The weight of the soil from 28 – 84 cm was used, along with the amount of DOC added at a flow rate of 220 mg DOC d^{-1} for 133 days, to find the total loading of DOC. The total loading of dissolved organic carbon (DOC) from 28 – 84 cm was 1.5 g C kg $soil^{-1}$ or 0.15% DOC, which would not account for the estimated increase of 0.6% DOC found within the columns. A previous experimental trial was run on the columns, with wastewater having a higher DOC content, and possibly accounted for the increase in OM content.

Total carbon ranged from 5 – 301 g C kg $soil^{-1}$ (Table 5.3). Significant interactions in TC were found for nitrogen*depth and salinity*plants*depth ($p < 0.001$). For the nitrogen*depth interaction, at a depth of 0 – 14 cm the 80%NH_4^+/20%NO_3^- treatment had a higher TC than the 100%NH_4^+ treatment (Figure 5.4). However, there was no significant effect of nitrogen at any other depth. From 0 – 14 cm, the low salinity, unplanted treatment had a significantly higher TC than all other salinity*plant combinations. At this depth, TC was dependent on both the salinity level and the presence or absence of plants (Figure 5.4). The significance of the salinity*plant combination was also dependent on the initial percentages of OM in the soil, as the low salinity treatment had a higher level of OM, which would contribute to a higher level of TC. Total C was significantly higher ($p < 0.001$) in wastewater treated columns versus control columns, an indication addition of wastewater increased TC levels.

Total N was only measurable from 0 – 28 cm and ranged from 0.89 – 22 g N kg soil^{-1} (Table 5.3). All other depths were below the detection limit of 0.5 g N kg soil^{-1}. Significant interactions in TN were found for salinity*plants*depth ($p < 0.001$). From 0 – 14 cm, only the low salinity, unplanted treatment was significant. This treatment had a significantly higher TN relative to all other treatments. Though the higher levels of OM in the low salinity treatments likely account for the higher TN from 0 – 14 cm, sorption of wastewater nitrogen to OM may also have contributed to the higher TN concentrations at this depth.

Bulk density averaged 1.46 (± 0.07) g cm^{-3} from 28 – 84 cm, 0.79 (± 0.18) g cm^{-3} from 14 – 28 cm, and 0.27 (± 0.15) g cm^{-3} for 0 – 14 cm below the surface (Table 5.3). Both the salinity*depth and plant*depth interactions were significant ($p < 0.001$). From 0 – 14 cm and 14 – 28 cm, the bulk density was significantly lower in the unplanted treatment versus the planted treatment. Bulk density was significantly lower in the low salinity treatment than the high salinity at the same depths (Figure 5.7). This was likely due to the differences in OM content of the surface soil applied to simulate field conditions. There were no significant differences in the bulk density from 28 – 84 cm. Bulk density would normally be expected to be lower in the planted columns, as plant roots (and therefore OM) lower bulk density (DeLaune et al., 1979; Baustian et al., 2009). However, the unplanted columns in this study had a higher OM content. The lower bulk density in unplanted columns and in the low salinity was explained by the presence of microbial mats that formed near the surface (0 – 28 cm) during the experiment. The microbial mats were most prevalent in the low salinity, unplanted columns and were very spongy and rose to an approximate height of 12 cm above the soil surface. Microbial mats are composed of a complex consortium of cohesive bacteria and are responsible for a wide variety of biochemical and ecological interactions. They are ubiquitous and found from marine and freshwaters to wetlands to soil to deep ocean hydrothermal vents and Antarctic ponds and sea ice

(Bender and Phillips, 2004; Rejmankova and Komarkova, 2005). The wide ranging redox gradients present in most mats help account for the high productivity seen in some mats (Bender and Phillips, 2004). The buoyancy often exhibited by microbial mats is due to the formation of gases within their matrix, which explained the increase in soil height and lower bulk densities seen in the low salinity, unplanted columns in this study.

5.3.2 Microbial Biomass Carbon

Microbial biomass C ranged from 0.02 – 23.4 g C kg soil^{-1} (Figure 5.8). The salinity *plants*depth interaction was significant for MBC ($p < 0.01$). Significant differences in MBC

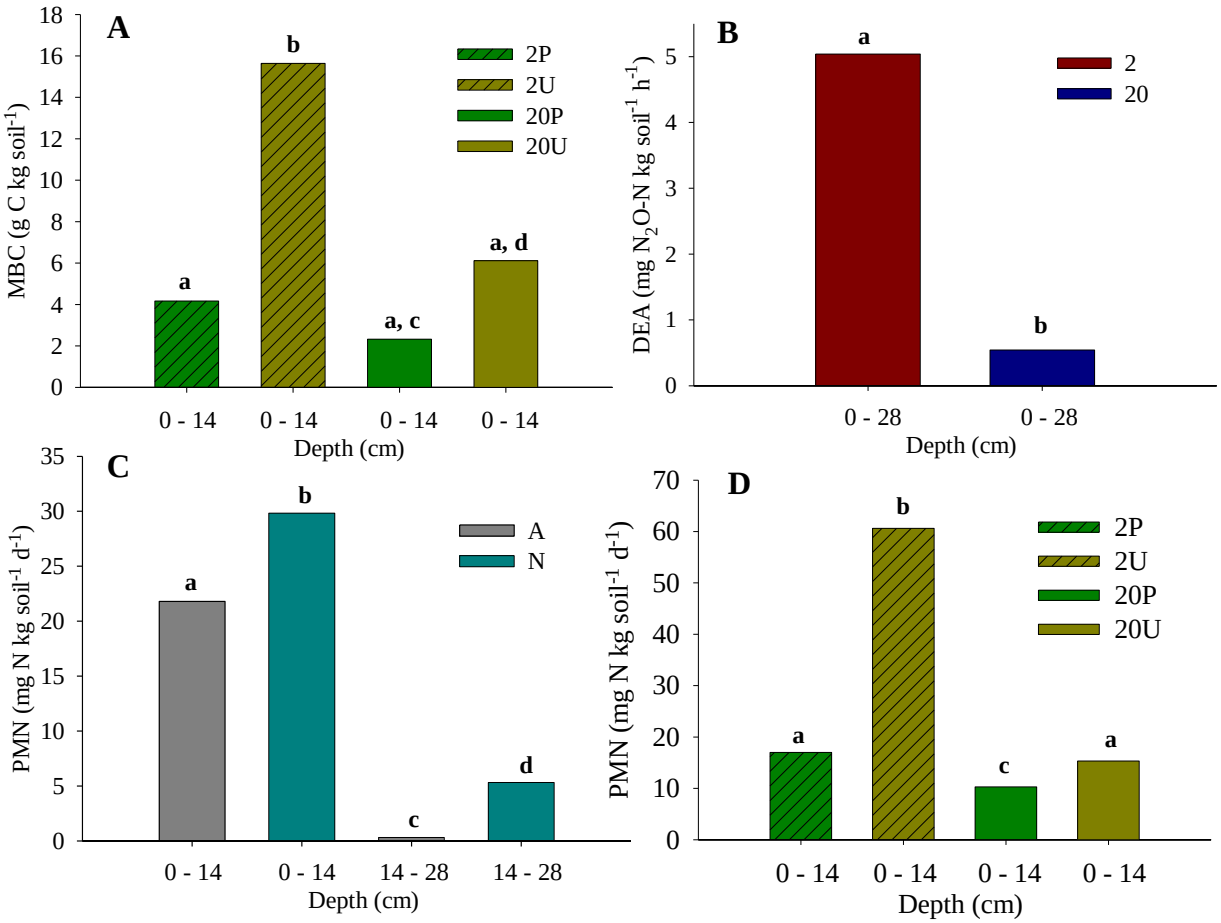


Figure 5.8 Significant statistical comparisons of microbial characteristics. Letters indicate treatment combinations with significant differences. Treatments are not compared to controls as comparison to controls was done with a different analysis. Only significant depths are shown, all depths not shown were found to have no significant difference. Graph A shows microbial biomass carbon MBC, B shows denitrifying enzyme activity (DEA), and C and D show potentially mineralizable nitrogen (PMN).

were present only from 0 – 14 cm. At this depth, the low salinity, unplanted treatment was significantly higher than other salinity*plants treatment combinations (Figure 5.8). Microbial biomass C was dependent upon both the salinity and plants, but generally the low salinity and unplanted treatments had a higher MBC from 0 – 14 cm. While productive, large microbial communities are generally associated with the rhizosphere in wetland soils and, therefore, also associated with plants, the higher MBC in this study was found in the surface of unplanted columns. The increased MBC present in the unplanted columns was due to the highly productive microbial mat found in the unplanted columns. The low salinity, unplanted columns had the most noticeable microbial mat and also had the highest levels of MBC. There is also some evidence suggesting higher salinities decrease MBC due to salinity-induced stress (Rietz and Haynes, 2003; Yuan et al., 2007a; Yuan et al., 2007b), accounting for the higher MBC found in the low salinity.

Overall, the highest levels of MBC were found in the top 0 – 14 cm (Figure 5.8) and were significantly higher ($p < 0.001$) in wastewater treated columns than control columns at this depth. Though MBC is not directly related to microbial activity, it does give some indication of the microbial presence. Thus, wastewater increased the levels of MBC and, subsequently, microbial activity. The higher levels of MBC being found near the surface indicated there was a higher microbial activity at the surface (more oxidized) and a lower activity at deeper depths where the environment was more reduced. Microbial activity and OM decomposition in reducing environments are generally much slower than in oxygenated environments. D'Angelo and Reddy (1999) found that carbon mineralization was three times slower in anaerobic environments, while White, et. al., (2001) found that nitrogen mineralization was six times slower. Other studies have also found that MBC is highest closer to the surface in various soil environments, including wetland and agricultural soils (Haines and Uren, 1990; White and

Reddy, 1999; Peacock et al., 2001). The higher levels of MBC are related to the high concentrations of TC and TN (which is also related to high OM content) also found at the column surface. Presence of MBC is related to DOC as DOC is a more readily available food source. Nutrient availability has a large impact on the size and activity of the microbial population. The availability of carbon and nitrogen plays a large role in influencing the size of the soil microbial biomass. Increases in readily available carbon sources (such as the injected wastewater) stimulate the growth and activity of the microbial pool (Anderson and Domsch, 1985; Schnurer et al., 1985). A C:N ratio of 3 – 5 is considered optimal for good microbial growth (Paul and Clark, 1996). The mean C:N ratio for this study was 15. Though this number indicates that nitrogen is limiting, large amounts of nitrogen are present due to incoming wastewater and microbial growth could still be high, as was seen from 0 – 14 cm in this study.

5.3.3 Denitrifying Enzyme Activity

Denitrification is important to the reduction of nitrogen loads from wetland wastewater treatment systems and, as such, the denitrifying enzyme activity provides a measure of the rate at which denitrification is occurring within these systems. Understanding the influences on denitrifiers will help to improve the denitrification potential in these systems as denitrifiers convert NO_3^- to N_2, thus removing nitrogen from the system. Because of the low number of data points above the DEA detection limit, data were grouped into two groups: 0 – 14 and 14 – 28 cm, and 70 – 84 cm and the sand layer. The majority of DEA occurred near the surface between 0 – 28 cm where DEA ranged from 0.01 – 22.6 mg N_2O-N kg^{-1} h^{-1} and was significantly higher ($p < 0.001$) in wastewater treated columns than control columns. From 70 – 84 cm/sand, the DEA was below 0.06 mg N_2O-N kg^{-1} h^{-1} for all treatments (Figure 5.5). The salinity interaction was significant for a log transformation of the data from 0 – 28 cm at $p = 0.09$, where the low salinity DEA was higher than the high salinity (Figure 5.8, data shown without log

transformation). A log transformation was used to ensure normality of the data. Though the difference was only moderately significant at p = 0.09, salinity showed an influence on denitrification rates occurring within the columns. Increased salinities are related to decreases in denitrification activity (Yoshie et al., 2004; Seo et al., 2008) and are likely why the low salinity treatment had a higher DEA rate from 0 – 28 cm.

Because the 80/20% NH_4^+/NO_3^- treatment received 20 mg N-NO_3^- L^{-1} and the 100% NH_4^+ treatment received no NO_3^-, the treatment receiving NO_3^- would be expected to have a significantly higher DEA (from 70 – 84 cm) than the treatment not receiving NO_3^-. Though the treatments were not significantly different, 100% of the DEA detectable was from 70 – 84 cm and occurred within the 80/20% NH_4^+/NO_3^- treatment (Figure 5.9). However, from 0 – 28 cm the percentage of DEA occurring between the two columns was nearly equal, with the 100% NH_4^+ treatment being slightly higher at 53%. This relationship is explained by nitrification/

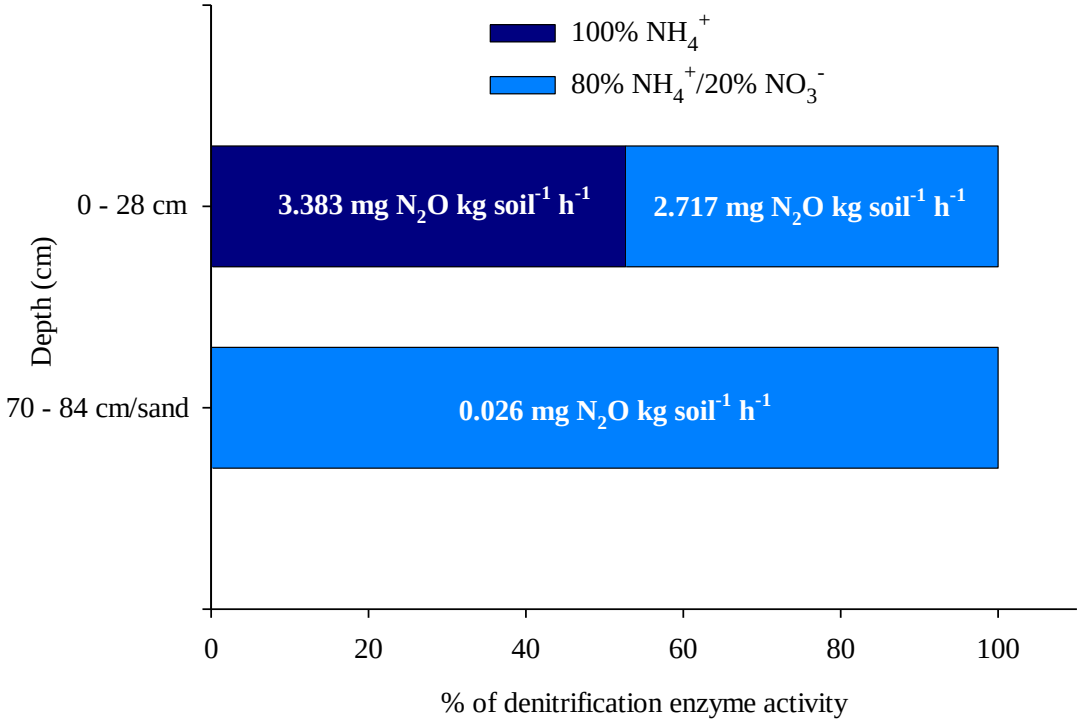


Figure 5.9 Percent of total denitrification enzyme activity (DEA) occurring in the two nitrogen wastewater treatments at 0 – 28 and 70 – 84 cm below the surface. Numbers within bars are mean DEA occurring at corresponding depth and wastewater treatment.

denitrification coupling occurring at the surface (Reddy and Patrick, 1984) as the wastewater NH_4^+ is converted to NO_3^- by nitrifying bacteria. High levels of NH_4^+ were present in all columns and would undergo little change in chemical form as the wastewater moved vertically up the column (though the anaerobic subsurface) until the surface was reached. The high levels of NH_4^+ reaching the surface (see Chapter 4) and introduction of oxygen at the topmost surface and rhizosphere would stimulate nitrification and, subsequently, denitrification, as NO_3^- diffused into anaerobic regions. Because of the close proximity of aerobic and anaerobic soil environments at the soil surface and within the rhizosphere, efficiency of nitrification and denitrification is increased. Though nitrification rates were not measured, the occurrence of denitrification within the 100% NH_4^+ treatment strongly suggests NH_4^+ is being converted to NO_3^- prior to denitrification

If the loading of NO_3^- is considered relative to the DEA measured within the subsurface, the nitrogen load was found to be higher than the nitrogen measured by DEA. The level of NO_3^- loaded was 0.14 mg N kg soil^{-1} h^{-1}, which was five times higher than the mean DEA measured at that layer (0.026 mg N kg soil^{-1} h^{-1}). Even considering the highest DEA measured at this depth (0.057 mg N kg soil^{-1} h^{-1}), the NO_3^- load was still considerably higher. A Br tracer study performed during the course of the study revealed the possibility of changing channelization patterns and frequent flow shifting in some regions of the columns. These changes in wastewater flow are believed to be due to temporary clogging caused by microbial growth. If these changes in flow did occur, some of the NO_3^- in the wastewater may have bypassed the bottom layers and moved into regions higher in the column. There was also the possibility that anammox or dissimilatory nitrate reduction to ammonium (DNRA) occurred. Anammox occurs under strongly reducing conditions where NO_3^- and NH_4^+ are used in conjunction by microorganisms to produce N_2 (Megonigal et al., 2004). Anammox bacteria would directly

compete for NO_3^- with denitrifiers, thus reducing the DEA that could occur. Dissimilatory NRA reduces NO_3^- to NH_4^+ as opposed to the N_2 gas formed during denitrification. Not as much is known about DNRA, but factors thought to favor it include, highly reduced soils (it consumes eight electrons as opposed to the five electrons consumed during denitrification) and high organic carbon content (Megonigal et al., 2004), both of which were indicative of the column conditions during this study.

The denitrification decrease with depth is consistent with other literature and is correlated to a number of influences, including nutrient availability (White and Reddy, 1999; Flite et al., 2001). The higher levels of OM present (Table 5.3) within the top 28 cm also contributed to the higher levels of denitrification occurring, as the bottom 14 cm only contained 2.41 ± 0.74% of OM (Burchell et al., 2007). The DEA was also positively correlated with TC ($p < 0.001$, $r = 0.69$) (Table 5.4). The correlation between DEA and TC was due to the influence of organic carbon on denitrification. Carbon is necessary for denitrification as it supports requirements for energy and cellular synthesis (Knowles, 1982; Burchell et al., 2007). Significant relationships between denitrification rates and water soluble organic carbon have been shown for a number of wetland soil types (D'Angelo and Reddy, 1999). Low levels of OM found in mineral soils, often used in constructed treatment wetlands, limit denitrification (Nichols, 1983; Burchell et al., 2007). Because the MUS is used in a natural marsh containing higher amounts of OM than

Table 5.4 Correlation matrix of organic matter (OM), microbial biomass C (MBC), total C (TC), denitrifying enzyme activity (DEA), and potentially mineralizable N (PMN) for column soils. All r values listed are significant at $p < 0.01$.

	OM	MBC	TC	DEA
MBC	0.73			
TC	0.82	0.84		
DEA	0.58	0.39	0.69	
PMN	0.78	0.89	0.94	0.63

would be found in most constructed treatment wetlands, denitrification should not be limited by a lack of carbon.

The range of DEA found in wetland systems is wide. Flite et al., (2001) found DEA levels ranging from 0 – 0.21 mg N_2O-N kg $soil^{-1}$ h^{-1} in a riparian wetland, White and Reddy (1999) from 0.004 – 7.75 in an Everglades Water Conservation Area, and Schipper and McGill (2008) from 0.035 – 1.410 in soil irrigated with dairy processing effluent. Hunt et al., has studied DEA in various different wetlands treating swine wastewater, including constructed wetlands treating swine wastewater with DEA ranging from 0.210 – 0.516 mg N_2O-N kg $soil^{-1}$ h^{-1} (Hunt et al., 2003), a riparian zone adjacent to a swine wastewater spray field ranging from 0.003 – 1.66 (Hunt et al., 2004), and a marsh-pond-marsh constructed wetland treating swine wastewater ranging from 0.06 – 1.13 (Hunt et al., 2006). The measurable DEA in this studied ranged from 0.001 – 22.6 mg N_2O-N kg $soil^{-1}$ h^{-1} and, if the two highest (of 10.15 and 22.6) are excluded, the highest is then 6.39 mg N_2O-N kg $soil^{-1}$ h^{-1}. Excluding the two highest, the range of our data is comparable to other DEAs found in the literature. The highest DEA from this study is more than double any DEA found in other literature and was found in one of the columns containing a microbial mat. It is possible the high productivity of microbial mats contributed to the high DEA found in this column, though differences in wastewater composition and other environmental factors may also have contributed to differences in the DEA levels found.

5.3.4 Potentially Mineralizable Nitrogen

Mineralization is an important process in the nitrogen cycle and converts organic nitrogen to inorganic forms, particularly NH_4^+. Because NH_4^+ is a nutrient of primary concern in many wetland wastewater treatment systems, understanding the potential mineralization possible is important for optimization of these systems. Rates of PMN ranged from -16.35 to 77.12 mg

$N\text{-}NH_4^+$ kg $soil^{-1}$ d^{-1} (Figure 5.6) and were significantly higher ($p < 0.001$) in wastewater treated columns than in control columns. In this study, both the salinity*plants*depth and nitrogen *depth interactions were significant for PMN ($p < 0.01$). The low salinity, unplanted treatment was significantly higher from 0 – 14 cm (Figure 5.8). The rate of PMN from 0 – 14 cm was found to be dependent upon both the salinity and plant effects, but not at any other depth. Higher salinities have been found to have a negative impact on the microbial activity related to the rate of PMN (Yuan et al., 2007a) and accounted for the significant differences in the PMN rates. For the nitrogen*depth interaction, both the 0 – 14 and 14 – 28 cm depths had a greater PMN rate for the $80\%NH_4^+/20\%NO_3^-$ treatment relative to the $100\%NH_4^+$ treatment. Nitrogen was not found to have a significant effect at any other depth. The significantly higher PMN rates in the $80\%NH_4^+/20\%NO_3^-$ treatment are likely due to higher TC levels also found for this treatment relative to the $100\%NH_4^+$ treatment. The rate of PMN was found to be positively correlated with TC ($p < 0.001$, $r = 0.94$) and MBC ($p < 0.001$, $r = 0.86$) (Table 5.4). Heterotrophic microorganisms can largely influence the availability of inorganic N. Wetland soil microbial biomass has been significantly correlated with the rates of N mineralization (Williams and Sparling, 1988; McLatchey and Reddy, 1998; White and Reddy, 2001). The correlation between TC and PMN further supported the higher PMN rates found within the $80\%NH_4^+/20\%NO_3^-$ treatment. The higher levels of MBC and TC from 0 – 14 cm also influenced the increased PMN rates of the same depth. This was also reflected by low PMN rates and low amounts of MBC and TC found at the lower depths (28 – 84 cm).

The range in N mineralization rates varies widely across different wetland soils. Nitrogen mineralization rates ranged from 0.084 – 0.650 mg $N\text{-}NH_4^+$ kg $soil^{-1}$ d^{-1} in wetland soils from two natural salt marshes in China (Bai et al., 2005), from 18.2 – 126 in wetland soil from a Water Conservation Area within the Everglades, Florida (White and Reddy, 2000), from -0.1 –

4.8 in constructed and natural wetland soils in Rhode Island (Duncan and Groffman, 1994), and from 1.75 – 13.83 in West African wetland soils used for rice production (Narteh and Sahrawat, 1997). Though these mineralization rates were not from soils treating wastewater, they do give an indication of the rates one would expect to find in natural soils. This study had mineralization rates ranging from -16.35 – 77.12 mg $N\text{-}NH_4^+$ kg $soil^{-1}$ d^{-1} which are similar to the rates found in other studies. The negative rates found by our study and Duncan and Groffman's can occur when immobilization (the assimilation of inorganic nitrogen into microbial biomass) is higher than mineralization, or microbes are taking up more NH_4^+ than they are releasing (Reddy and DeLaune, 2008).

5.3.5 Potential Impacts for Field Implementation

One possible explanation for the higher microbial activity (as measured by MBC, DEA, and PMN) seen in this study under the low salinity is the influence of salinity on microbial processes. Increases in salinity have been shown to lead to decreases in the microbial biomass, rate of denitrification, and rate of nitrogen mineralization (Laura, 1977; Seitzinger et al., 1991; Rysgaard et al., 1999; Sardinha et al., 2003; Wichern et al., 2006; Yuan et al., 2007a; Seo et al., 2008). Agriculture practices resulting in increased salinity led to a decrease in the size and activity of the soil microbial biomass as well as a decrease in nitrogen mineralization (Yuan et al., 2007a). Seitzinger, et al., (1991) showed an indirect salinity effect on denitrification caused by a reduction in nitrification rates, which was due to reduced availability of exchangeable NH_4^+. Higher salinities are known to increase physiological stress in microbes, which could lead to a decrease in microbial diversity (de Franca et al., 2000; Yoshie et al., 2004; Grommen et al., 2005). Yoshie, et. al., (2004) has shown that salinities near that of seawater decrease the nitrite reductase gene (required for denitrification) diversity in wastewater treatment systems. Loss of

genetic diversity can lead to reduced physiological diversity, robustness and resilience, and a greater susceptibility to environmental perturbations.

Though salinity may have an impact, the properties of the soil into which wastewater is injected is important. A majority of the soil characteristics measured were higher for the low salinity treatment (Table 5.3). A higher OM and TC content would explain a higher MBC as well as increased rates of DEA and PMN. The initial OM content (from 0 – 14 cm depth) of the columns for the low salinity was 77%, whereas the high salinity surface soil was 24%. The higher OM content for the low salinity explains why the low salinity treatment soil parameters were higher from 0 – 14 cm. This study showed increased microbial activity occurred at depths in which a higher OM content was present. Because the MUS utilizes natural wetlands, the amount of OM should be higher than is typically found in constructed treatment wetlands (Nichols, 1983; Burchell et al., 2007). Wichern, et al., (2006) found that amendments of OM counteracted the negative impact of increased salinity. Installing the MUS within marsh ecosystems that have higher amounts of OM and readily available nutrients will help to ensure abundant microbial activity, which can reduce the nutrient load of the wastewater on the surrounding environment. Though once wastewater addition to the subsurface is initiated, nutrients should not be limiting for microbial growth.

Denitrification is the primary process which permanently removes nitrogen from the MUS, as wastewater is injected into the subsurface (i.e., anaerobic zone). Quick consumption of NO_3^- indicates that any conversion of NH_4^+ to NO_3^- before injection will result in rapid treatment of nitrogen within the MUS as NO_3^- is consumed within anaerobic zones, provided enough C is present. Alternatively, if oxygen could be introduced into various regions of the subsurface, this could potentially lead to an increase in nitrification and, therefore, denitrification and a loss of nitrogen from the system. Any NH_4^+ reaching the surface would be treated via nitrification/

denitrification coupling through closeness to the surface or plant roots. Available NH_4^+ would be nitrified to NO_3^- in aerobic regions and some NO_3^- would then diffuse into nearby anaerobic regions where it could be subsequently denitrified and lost from the system. However, it is not desirable for untreated wastewater to reach the surface, as this would defeat the purpose of the MUS, which is to treat wastewater before it reaches the surface and then contaminates nearby waterbodies.

5.4 Conclusion

This study indicated an increased knowledge of the microbial community and, influences thereon, can lead to important improvements in wastewater treatment. Salinity was found to have a significantly negative impact on MBC, DEA, and PMN rates. Higher amounts of OM and TC were also significantly correlated with increased MBC, DEA, and PMN rates. Thus, it is important the MUS be installed in marshes that have significant quantities of OM available to increase initial microbial processes until wastewater injection can provide enough nutrients to be sustainable. Introduction of wastewater significantly increased microbial growth at the surface of columns and, subsequently, lead to increases in nitrification, denitrification, and nitrogen mineralization, as estimated by DEA and PMN. If wastewater were to reach the surface, nitrification/denitrification coupling would become important for treating NH_4^+ within the MUS. Plants could increase nitrification/denitrification coupling through the rhizosphere and have a positive impact on nitrogen removal. Within the subsurface, NO_3^- was converted to N_2 via denitrification. Thus, it would be beneficial for NH_4^+ to be converted to NO_3^- before injection so that NH_4^+ could be removed via denitrification. A better understanding of the linkages between the microbial community and ecosystem function can lead to improvements in the use of wetlands for wastewater treatment and for assessing the effects of perturbations on wetland ecosystems, such as environmental and pollutant variables (Cordova-Kreylos et al., 2006).

Therefore, consideration of the microbial transformations occurring and facilitating these transformations to improve wastewater treatment is vital to improve the long-term sustainability of the MUS.

CHAPTER 6: AMMONIUM SORPTION AT VARIOUS SALINITIES UNDER AEROBIC AND ANEROBIC CONDITIONS

6.1 Introduction

A primary component of domestic wastewater is nitrogen, which is present in various forms. Ammonium (NH_4^+) is the main nitrogen form of concern in many wastewater treatment systems. If NH_4^+ is released into nearby waterbodies, it can act as a toxin to some aquatic species (Camargo and Alonso, 2006; Paerl, 2006; Burkholder et al., 2007) and as a pollutant. Excessive concentrations of NH_4^+ in surface waters contributes to eutrophication, which can lead to decreases in dissolved oxygen levels in waterbodies. Decreases in dissolved oxygen can, in turn, create problems for aquatic species that rely on oxygen for optimal health (Camargo and Alonso, 2006; Burkholder et al., 2007). Thus, treatment of NH_4^+ before it can be introduced into the environment is vital.

The treatment of NH_4^+ in wastewater can be achieved by utilizing soils to remove NH_4^+. Zeolite soils are commonly used for this purpose, as they are selective for NH_4^+, but other soils have also been used (Hedstrom, 2001; Fernando et al., 2005; Ji et al., 2007; Karadag et al., 2007). Ammonium can also be sorbed onto wetland soils and organic matter associated with these soils. There are two types of NH_4^+ sorption to clay minerals: an ion exchange reaction that occurs on the surface of negatively charged clays (exchangeable NH_4^+) and sorption into the interlayers of the clay structure in certain clays (fixed NH_4^+). Ammonium also sorbs to organic matter due to the natural negative charge organic matter carries. Generally, the higher the organic matter content, the higher the NH_4^+ sorption (Rosenfeld, 1979; Hou et al., 2003; Fernando et al., 2005).

Though the negative charges of the soil attract NH_4^+, other cations, such as K^+, Na^+, Ca^{2+}, Fe^{2+}, and Mg^{2+}, are also attracted with varying strengths and compete with NH_4^+ for adsorption

sites. The degree to which the soil retains NH_4^+ over other cations plays an important role in how soon the soil will become saturated with respect to NH_4^+. Ammonium sorption is also influenced by pH, salinity, temperature, cation exchange capacity (CEC), anions, and other cations (Dalal, 1975; Gardner et al., 1991; Seitzinger et al., 1991; Evangelou, 1998; Rysgaard et al., 1999; Demir et al., 2002; Hou et al., 2003). As the pH increases, NH_4^+ changes form to NH_3, which volatilizes and, therefore, less NH_4^+ is sorbed. For optimum NH_4^+ sorption, the pH should be below 7 (Evangelou, 1998; Demir et al., 2002). An increase in temperature will increase the exchange capacity of a soil, thus increasing NH_4^+ sorption (Demir et al., 2002). Salinity has a profound effect on NH_4^+ sorption, which decreases with increased salinity (Gardner et al., 1991; Seitzinger et al., 1991; Rysgaard et al., 1999; Hou et al., 2003). There is also an increased NH_4^+ desorption with increasing salinity, which is partially due to increased competition with other cations as salinity is increased (Rysgaard et al., 1999). Anions present in seawater also partially neutralize the charge of NH_4^+ ions, as anions form ion pairs with NH_4^+, and thus reduce the sorption of NH_4^+ to sediment particles (Dalal, 1975; Gardner et al., 1991; Rysgaard et al., 1999). An anion that is strongly sorbed by soil (i.e., PO_4^{3-}) may increase the sorption of its associated cation (in this case, NH_4^+). However, irrespective of the anion present, sorption increases with increasing cation exchange capacity (CEC) (Dalal, 1975).

Coastal wetland environments face difficulties treating domestic wastewater using conventional wastewater treatment systems (i.e., septic systems). These systems do not work well under the conditions present, such as high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater. The marshland upwelling system (MUS) was developed as an alternative wastewater treatment system to address these special issues (Stremlau, 1994; Watson Jr. and Rusch, 2001, 2002; Richardson et al., 2004; Richardson and Rusch, 2005; Fontenot et al., 2006; Evans and Rusch, 2007a, b). The MUS is designed to utilize

the prevalent natural wetlands along coastal areas. It consists of a collection/distribution tank, injection pump, programmable timer, injection well, and saturated subsurface soils (Figure 6.1). An effectively operating system is dependent upon the native groundwater salinity, the injection frequency and flow rate, and the natural filtering properties and microbial transformation processes of the native soil matrix.

Conditions within the MUS are conducive to retention of NH_4^+ (until saturation is reached) because, other than anammox (anaerobic ammonium oxidation) and microbial immobilization, there is no microbial process which permanently removes NH_4^+ from the system. Because NH_4^+ is the main form of nitrogen being injected by the MUS, there is some concern that, over time, the soil may become saturated with NH_4^+ and, therefore, reduce the ability of the system to remove nitrogen. Imperative to the knowledge of the nitrogen processes and transformations occurring within the MUS is an understanding of the sorption capacity of the soil under the specific conditions that exist within the system. The objectives of this study were to determine the effects of: 1) varying salinities and 2) aerobic and anaerobic environments on the sorption of NH_4^+ in wetland soils.

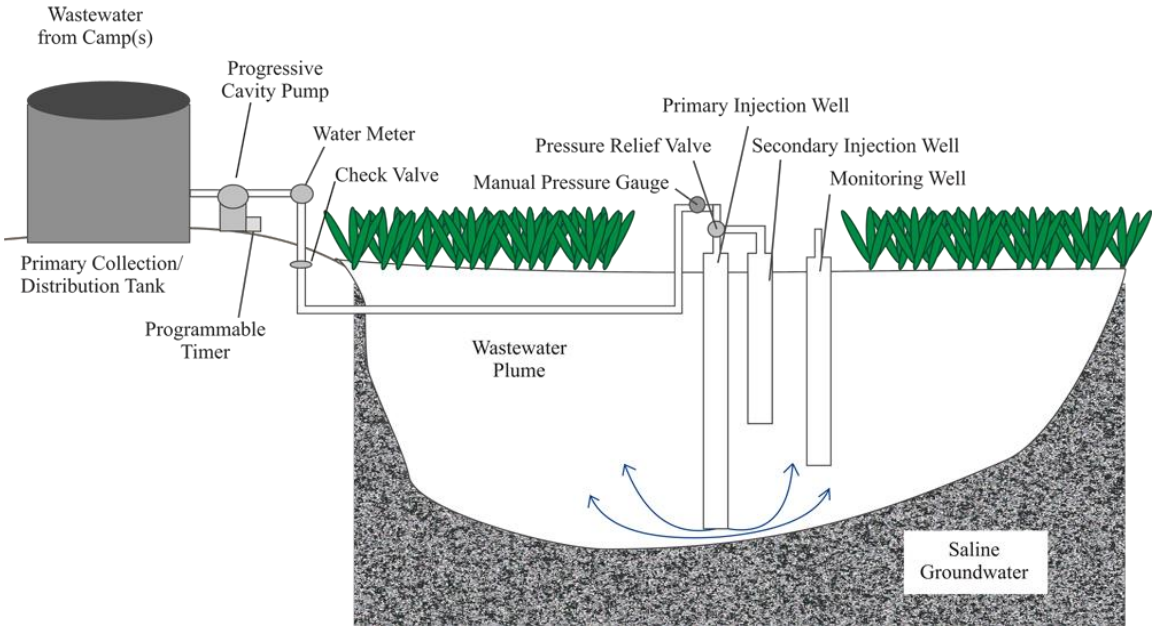


Figure 6.1 Generic schematic of the marshland upwelling system.

6.2 Materials and Methods

6.2.1 Experimental Setup

To better understand the sorption of NH_4^+ under the conditions found within the MUS, a more controlled sorption study was performed. Sorption of NH_4^+ was evaluated under aerobic and anaerobic conditions at salinities of 2, 10, and 20 parts per thousand (‰) (Table 6.1) at initial NH_4^+ concentrations of 0, 10, 20, 40, 60, 80, 100, and 120 mg NH_4^+-N L^{-1}. Wet soil was weighed out into 30 mL centrifuge tubes for a 10:1 liquid (i.e., NH_4^+ solution) to dry soil ratio. Wet soil, with no pH adjustment, was used instead of dry to ensure sorption was as close to experimental conditions as possible. Each treatment combination was weighed out in triplicate for a total of 144 samples. Ammonium solutions were made up in the appropriate salinity. Solutions used for the aerobic samples contained 0.6 g L^{-1} TCMP (2-chloro-6 (trichloromethyl) pyridine) (Hach, Loveland, CO) for nitrification inhibition. Aerobic samples were allowed to be open to the air and no effort was made to remove oxygen. All solutions used for anaerobic samples were bubbled with 99.99% O_2-free N_2 gas to ensure no oxygen was present. Centrifuge tubes for anaerobic samples had sealed septa caps and were flushed with the same N_2 gas. Anaerobic samples were kept closed and samples pulled from tubes with a needle to ensure oxygen was not introduced. Samples were shaken on a reciprocal shaker for 24 h after addition of NH_4^+ solutions for sorption. After 24 h, samples were centrifuged and NH_4^+ solution was

Table 6.1 Concentrations of dry salts used for each salinity treatment.

	2‰	10‰	20‰
Salt	Concentration (g L^{-1})		
$NaHCO_3$	0.03	0.13	0.25
$MgCl_2 \cdot 6H_2O$	0.62	3.13	6.25
$CaCl_2 \cdot 2H_2O$	0.09	0.43	0.85
K_2SO_4	0.002	0.01	0.02
KCl	0.04	0.15	0.29
NaCl	0.75	10.00	20.00

removed and filtered. The samples were shaken an additional 24 h after addition of the appropriate salt solution (with no added NH_4^+) for desorption. All samples were filtered (0.45 µm filters), preserved at pH < 2, and analyzed within 48 h for NH_4^+ using a Seal Analytical AQ2 Automated Discrete Analyzer following EPA method 350.1 (USEPA, 1993).

6.2.2 Soil Analyses

Soil was collected from a salt marsh in Port Fourchon, Louisiana, and then homogenized and sterilized. The soil was sterilized to prevent the introduction of saltmarsh microorganisms into a lower salinity treatment in a column laboratory study (Chapters 4 and 5). Soil used in this sorption study was taken from the same batch of soil used in the laboratory study, but underwent no wastewater introduction. Sand, silt, and clay fractions were measured using sieve (ASTM C117, C136) and hydrometer analyses (ASTM D422) (ASTM, 1995). X-ray diffraction analysis was performed in the Louisiana State University Department of Geology X-ray crystallography laboratory using a Bruker/Siemens D5000 automated powder X-ray diffractometer with Rietveld analysis software. Organic matter was measured as loss on ignition (Sparks, 1996). Cation exchange capacity was determined following Sparks (1996).

6.2.3 Data Analyses

SAS® software (2009) and SigmaPlot® software (2002) were used to analyze data. All statistical tests were performed at a significance level of $\alpha = 0.05$. Isotherms were constructed by using the mean of three replicates and plotting the mass of NH_4^+-N sorbed per mass of soil versus the NH_4^+-N concentration in solution at equilibrium. The mean of replicates was used to fit isotherms in order to reduce experimental variability. The sorption isotherms used were:

the linear equation

$$q = K_d C_{eq} \tag{6.1}$$

where q is the mass of solute sorbed per dry unit weight of sorbent (mg kg^{-1}), C_{eq} is the equilibrium concentration of solute after sorption (mg L^{-1}), and K_d is the distribution coefficient (L kg^{-1}); the Freundlich equation

$$q = KC_{eq}^{\ N} \tag{6.2}$$

where q and C_{eq} are as above, K is the Freundlich constant (L kg^{-1}), and N is the heterogeneity factor (unitless); and the Langmuir equation

$$q = \frac{\alpha\beta C_{eq}}{1 + \alpha C_{eq}} \tag{6.3}$$

where q and C_{eq} are as above, α is the adsorption constant related to binding energy (L mg^{-1}), and β is the maximum amount of solute that can be sorbed by the solid (mg kg^{-1}). The sorption isotherms used were chosen as they are commonly found in the literature allowing for comparisons to other studies.

In order to determine significant differences between treatments, each set of sorption data was fit to the Langmuir equation. Individual sorption data sets were fit to the Langmuir equation so that replicate q's could be estimated at a specific C_{eq}. The estimated C_{eq}'s were then compared using a two-way ANOVA to determine significant differences for the salinity and oxygen treatments at each C_{eq}. A two-way ANOVA was also used to analyze the % NH_4^+ desorbed (desorbed/sorbed). Due to high variability in the data, percentages were averaged for each treatment combination and compared statistically.

6.3 Results and Discussion

6.3.1 Ammonium Isotherms

The soil was primarily composed of sand (78%), with silt composing 18% and clay 4% (Table 6.2). Organic matter content of the soil was 1.3%. Salinity (2, 10, and 20‰), oxygen level (aerobic and anaerobic), and initial NH_4^+ concentration (0, 10, 20, 40, 60, 80, 100, and 120

Table 6.2 Soil properties.

Property	
sand (%)	78
silt (%)	18
clay (%)	4
USDA classification	loamy sand
organic matter (%)	1.3
CEC (centimoles of cation charge kg soil^{-1})	14.5

mg N-NH_4^+ L^{-1}) were imposed on a total of 144 samples. Triplicate samples were averaged and the mean plotted as NH_4^+ equilibrium concentration (C_{eq}) versus NH_4^+ sorbed to soil (q). Data points appearing to be outliers were tested using the *DFFITS* option in *SAS*® (Mendenhall et al., 1999). Points determined to be outliers were excluded and remaining points were used to fit the data using the linear, Freundlich, and Langmuir equations. Each salinity/oxygen treatment combination was analyzed for fit individually for each sorption isotherm. All equations showed a good fit ($r^2 > 0.83$) to the data (Table 6.3), except for the 10‰ data (not shown). The data for the 10‰ treatment (Figure 6.2) could not be evaluated due to negative numbers found at higher NH_4^+ concentrations. Therefore, the data for the 10‰ treatment was not included in these analyses. Previous experiments, looking at different parameters, have observed the same trend

Table 6.3 Coefficients and r^2 values for linear, Freundlich, and Langmuir equations for sorption data.

Coefficient	Treatment			
	2‰ aerobic	2‰ anaerobic	20‰ aerobic	20‰ anaerobic
Linear				
r^2	0.9713	0.8367	0.8618	0.9407
K_d (L kg^{-1})	2.33	2.08	0.72	0.46
Freundlich				
r^2	0.9876	0.8799	0.9228	0.9609
K (L kg^{-1})	5.60	8.34	2.80	1.33
N (unitless)	0.80	0.68	0.69	0.77
Langmuir				
r^2	0.9878	0.8609	0.9488	0.9526
α (L mg^{-1})	0.0055	0.0087	0.0105	0.0047
β (mg kg^{-1})	604	402	132	142

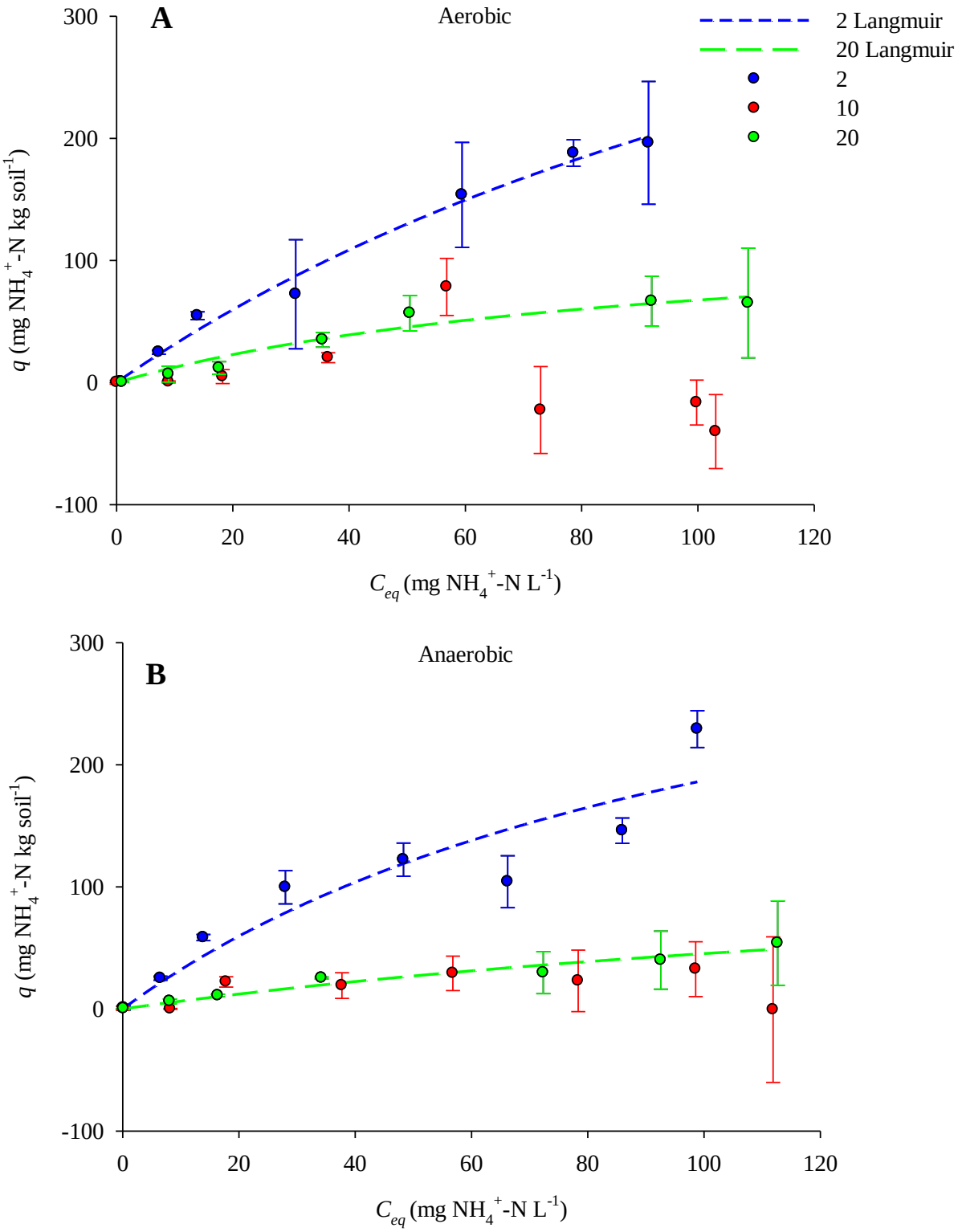


Figure 6.2 Plots of 2, 10, and 20‰ salinity sorption data for aerobic (graph A) and anaerobic treatment (graph B). Data points represent mean of data points and the standard deviation is indicated by error bars. Estimated Langmuir equations are shown for 2 and 20‰.

for data to breakdown in the salinity range between fresh and saltwater (8 – 12‰) (K. A. Rusch, personal communication). The reason for this breakdown is unclear and no literature could be found discussing any salinity effects between 8 – 12‰. However, it is theorized the breakdown may be due to physical or molecular interferences that occur in this salinity range.

Even though all equations gave a good fit (r^2 value > 0.84), the Langmuir model was used to describe the data as it gave a slightly better fit for most treatments. The Langmuir model also describes more realistic conditions. The sorption isotherms used were chosen as they are commonly found in the literature allowing for comparisons to other studies. However, there are limitations associated with each model. The linear model, while the simplest and easiest model to use numerically, is not a logical model to use as the model provides an equation in which there are no limits on the amount of NH_4^+ that can be sorbed. The Freundlich model is purely an empirical model, is not thermodynamically consistent, and the mass of solute sorbed does not reach an upper limit (Thornton et al., 2007; Zheng et al., 2008). Though the Langmuir model may have limitations (i.e., it assumes sorption sites are homogeneous), this model is often used to describe NH_4^+ sorption data because it imposes a limit on sorption (Karadag et al., 2007; Thornton et al., 2007) and provides an adequate fit of the data for this study.

The amount sorbed decreased with increasing salinity for both the aerobic and anaerobic treatments (Figure 6.2). The influence of salinity on sorption is expected, because as the salinity increases the number of cations (i.e., molecular competition) also increases and, therefore, less NH_4^+ is sorbed to the soil (Seitzinger et al., 1991; Rysgaard et al., 1999; Hou et al., 2003). These observations were backed up by statistical analysis of the data, which shows the low salinity had a significantly higher amount of NH_4^+ sorbed for each C_{eq} concentration (Figure 6.3). No significant effects of oxygen level were seen on NH_4^+ sorption. Oxygen levels had no significant effect on sorption at any C_{eq} concentration. Though oxygen levels had no significant effect on

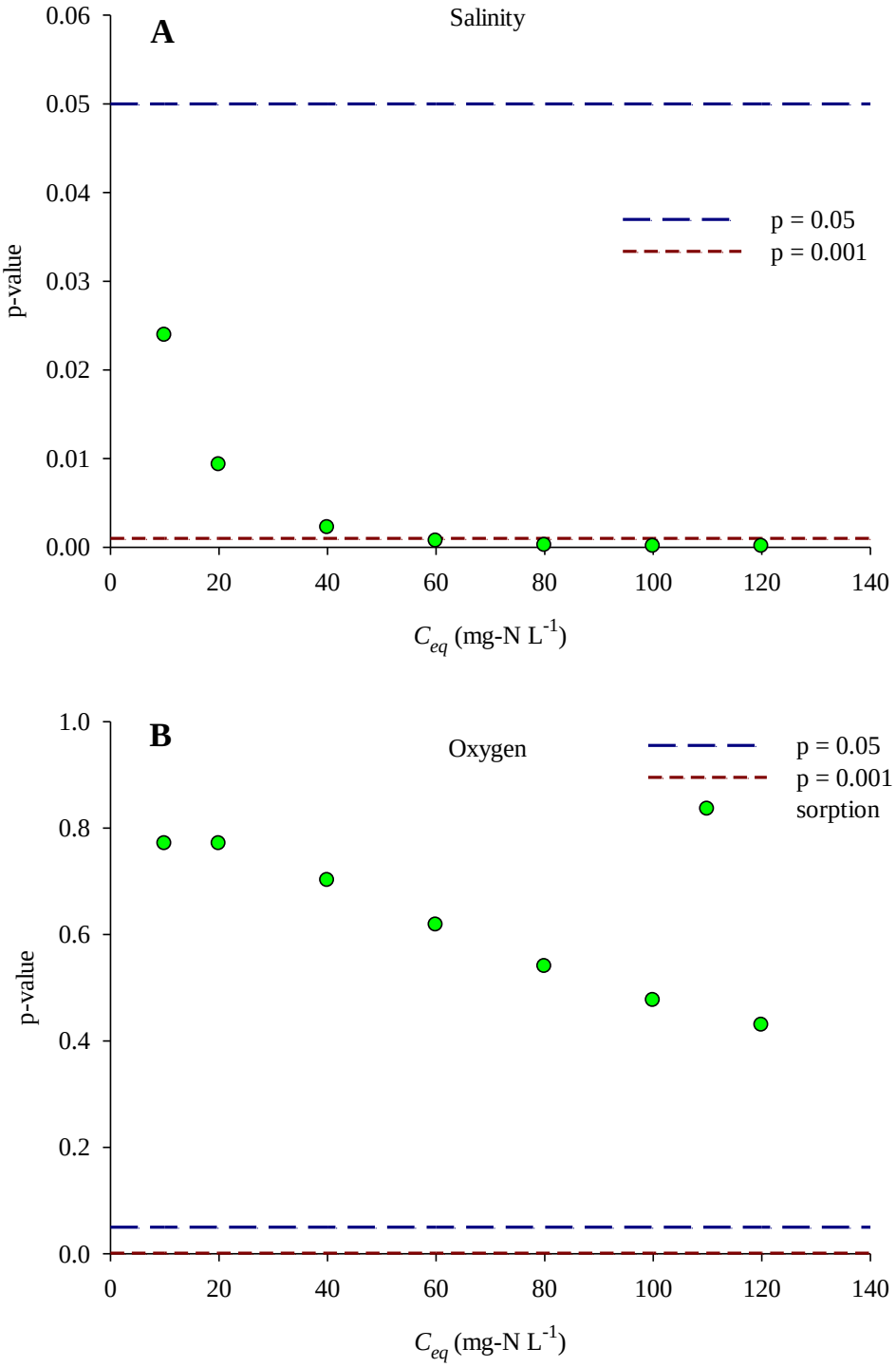


Figure 6.3 The p values plotted by estimated C_{eq} concentration for salinity and oxygen effects on sorption and desorption. C_{eq} concentrations were estimated from Langmuir equations fitted to each set of experimental data resulting in replicates for each C_{eq} concentration. The estimated C_{eq}'s were then analyzed in a two-way ANOVA to determine significance for each treatment. The p values are plotted to show the change in significance as C_{eq} increases.

sorption, under anaerobic conditions there is increased competition from Fe^{2+} and Mn^{2+}. This could lead to a lower sorption capacity under anaerobic conditions as Fe and Mn are reduced from Fe^{3+} and Mn^{4+} (solid forms) to Fe^{2+} and Mn^{2+} (more soluble forms) and are preferentially adsorbed relative to NH_4^+ (Phillips, 2001).

The sorption seen in this study is comparable to previous studies that were performed using a variety of materials for sorbents (Table 6.4). The studies cannot be compared directly as experimental conditions varied widely and sorption results are dependent on the length of time solute and sorbent are in contact, quantity of sorbent present, quantity of solute present, pH, and temperature (Shen et al., 1997; Wang and Alva, 2000; Hedstrom, 2001; Thornton et al., 2007). The amount of NH_4^+ sorbed (q) at C_{eq}s of 100 and 80 mg NH_4^+-N L^{-1} were calculated using the Langmuir values observed from each study. These concentrations were chosen to represent the NH_4^+ concentrations used in a laboratory column study (Chapters 4 and 5) designed to study the nitrogen treatment effectiveness of the MUS in more detail than previous studies (Turriciano, 2005; Fontenot et al., 2006). Though the studies cannot be directly compared, it was observed that the soil from this study achieved a lower amount of sorption than most other studies. The low sorption was likely partially due to the high sand content (78%) of the soil. The studies with the most similar results were those using the Kennebec, Haynie, and rice paddy soils (Fernando et al., 2005; Liu et al., 2008). In comparison to the soil from this study, the Kennebec soil contained 20% sand, while the Haynie soil contained 34% sand (Fernando et al., 2005). The studies having a higher sorption capacity used soils and particles that are known to have a high sorption specificity for NH_4^+ (Hedstrom, 2001; Helminen and Paatero, 2006; Ji et al., 2007; Karadag et al., 2007; Thornton et al., 2007). In comparison, sand contents for some of the zeolites used ranged from 44 – 74% (Karadag et al., 2006; Gunay, 2007; Lei et al., 2008; Zheng et al., 2008). The soil from this study found a low sorption capacity for NH_4^+.

Table 6.4 Comparison to other sorbent types for ammonium sorption using the Langmuir isotherm.

Sorbent Type	Experimental Conditions		α ($L\ mg^{-1}$)	β ($g\ kg^{-1}$)	q at C_{eq} (mg NH_4^+-N L^{-1}) 80	100	Reference
Natural Turkish clinoptilolite zeolite	Media loading: 10 g L^{-1} Temperature 25°C NH_4^+-N concentration range 25-150 mg L^{-1}		0.029	8.121	5.67	6.04	(Karadag et al., 2006)
Natural Bigadic clinoptilolite zeolite	Media loading: 4 g L^{-1} Temperature 23°C, pH 6.5 NH_4^+-N concentration range: 5 - 150 mg L^{-1}		0.061	15.207	12.62	13.07	(Gunay, 2007)
MesoLite media (synthetic zeolite)	Media loading: 5 g L^{-1} Temperature 20°C NH_4^+-N concentration range: 0 - 2000 mg L^{-1}		0.0658	27.781	23.35	24.12	(Thornton et al., 2007)
Microwave-treated natural Chinese zeolite	Media loading: 3 g L^{-1} Temperature 25°C, pH 6.5 NH_4^+-N concentration range: 0 - 200 mg L^{-1}		0.23	13.74	13.03	13.17	(Lei et al., 2008)
Kennebec soil	Media loading: 100 g L^{-1} NH_4^+-N concentration range: 75 - 2085 mg L^{-1}		0.0043	0.909	0.233	0.273	(Fernando et al., 2005)
Haynie soil			0.0230	0.217	0.141	0.151	
Rice paddy clay (no fertilizer)	Media loading: 200 g L^{-1} Temperature 25°C, pH 5.5 NH_4^+-N concentration range: 0 - 300 mg L^{-1}		0.016	0.303	0.170	0.186	(Liu et al., 2008)
Zeolite 13x	Media loading: 16 g L^{-1} Temperature 25°C, pH 7.0 NH_4^+-N concentration range: 5 - 400 mg L^{-1}		0.44	8.61	8.37	8.42	(Zheng et al., 2008)
Sulfonated polystyrene silica gel	Temperature 25°C, pH ≤ 7.0 NH_4^+-N concentration range: 0.4 - 140 mg L^{-1}		0.213	8.826	8.34	8.43	(Helminen and Paatero, 2006)
Coastal soil	Media loading: 10 g L^{-1}	2‰ aerobic	0.0055	0.604	0.185	0.214	This study
	NH_4^+-N concentration range: 0 - 120 mg L^{-1}	2‰ anaerobic	0.0087	0.402	0.165	0.187	
		20‰ aerobic	0.0105	0.132	0.060	0.068	
	Temperature: 24°C	20‰ anaerobic	0.0047	0.142	0.039	0.045	

As sorption is likely the primary method of NH_4^+ removal within the subsurface, the type of soil present in the MUS will be important for the sorption of NH_4^+. Soils containing higher amounts of clay and organic matter will sorb more NH_4^+ than soils containing more sand (i.e., quartz), as quartz is mostly inert and contributes little to sorption processes (Pierzynski et al., 2005). Some soils have more sorption sites present than others due to the nature of their chemical makeup. Generally the higher the organic matter content, the higher the NH_4^+ sorption (Rosenfeld, 1979; Hou et al., 2003; Fernando et al., 2005). Organic matter and, subsequently, organic carbon would be increased within the MUS as wastewater is injected into the subsurface. Increased amounts of dissolved organic carbon (DOC) from wastewater have been shown to increase the NH_4^+ sorption capacity of a soil (Fernando et al., 2005). Fernando, et al., (2005) found that DOC from swine wastewater resulted in a significantly higher sorption of NH_4^+ over a synthetic wastewater solution. For every 250 mg L^{-1} increase in DOC there was a 20 – 30% increase in NH_4^+ sorption. They proposed an increase in NH_4^+ sorption was due to NH_4^+-DOC complexes that sorbed to soil and had a higher retention relative to a simpler matrix (Stevenson, 1994; Fernando et al., 2005). The correlation between organic matter and carbon to the sorption of NH_4^+ is further supported by Chung and Zasoki (1994), who found an addition of organic matter increased the preference for NH_4^+ over K^+ and Burge and Broadbent (1961), who found a positive correlation between chemically fixed NH_4^+ and the percent of soil carbon.

The sorption of NH_4^+ within the subsurface of the MUS could also be influenced by changes in the pH, salinity, and redox potential caused by the introduction of wastewater. Changes in the pH of soils can lead to changes in the sorption of NH_4^+. Various researchers have found a pH between 6 – 8 to be the optimal range for NH_4^+ sorption (Hedstrom, 2001; Fernando et al., 2005; Thornton et al., 2007). At lower pHs, competition from H^+ ions reduces NH_4^+ sorption and at higher pHs, NH_4^+ is converted to NH_3 (Hedstrom, 2001; Thornton et al., 2007).

A slight increase in pH of the groundwater from fields sites (pH = 6.69) to background conditions (6.35) was observed and was likely due to the influence of injected wastewater pH (7.51). The influence of pH, however, would likely be minimal as the increase in pH was minimal and both the initial and final pH of the groundwater was within the optimal sorption pH range of 6 – 8. Increases in salinity negatively impact the sorption of NH_4^+ as an increase in the number of cations at higher salinities results in competition for sorption sites (Gardner et al., 1991; Seitzinger et al., 1991; Rysgaard et al., 1999; Hou et al., 2003). Introduction of wastewater into the groundwater present within the MUS will likely result in a decrease of salinity as the injected wastewater has a lower salinity than the native (i.e., near coastal) groundwater. Thus, sorption of NH_4^+ could be increased with the addition of wastewater. Organic nitrogen mineralization leads to an increase in NH_4^+ concentrations under anaerobic conditions because the microorganisms that perform the process have lower N requirements than microorganisms under aerobic conditions, thus leading to an accumulation of NH_4^+ under anaerobic conditions (Moore et al., 1992; Phillips, 2001; White and Reddy, 2001). Data from one MUS field site found the redox potential nearest to the injection site to be less reducing than soil at a greater depth. Introduction of small amounts of oxygen might lead to a slight increase in the accumulation of NH_4^+ due to mineralization and, subsequently, reduce the competition for sorption sites. Differences in soil type, organic matter concentration, pH, salinity, and redox potential between sites will affect the ability of the MUS to treat NH_4^+ via sorption.

The percentage of NH_4^+ desorbed from soil saturated with NH_4^+ was calculated and ranged from 41 to 91% (Table 6.5). There was a slight trend for percent desorbed to increase with increasing NH_4^+ concentration. The low salinity (overall mean of 63 ± 14%) tended to have a lower percentage of NH_4^+ desorbed relative to the high salinity treatment (73± 15%). For both the aerobic and anaerobic treatments, the low salinity had a lower percentage desorbed. A higher

Table 6.5 Percentage of NH_4^+ desorbed from soil after NH_4^+ saturation. Mean percentages ± standard error (SE) are presented in bold. Some numbers could not be calculated due to errors in the raw data and were not included.

Initial NH_4^+	Percentage Desorbed (%)			
(mg L^{-1})	2‰ aerobic	2‰ anaerobic	20‰ aerobic	20‰ anaerobic
10	79	41	59	40
20	68	41	88	77
40	67	50	75	91
60	45	58	74	--
80	66	82	56	91
100	78	73	84	81
120	75	57	63	68
mean	**68 (± 11)**	**57 (± 15)**	**71 (± 12)**	**75 (± 19)**

desorption at higher salinities is expected as there are more cations present to compete for sorption sites, thus, more NH_4^+ will be desorbed as competing cations take its place. The effect of oxygen status is less clear. The aerobic treatment had a higher overall mean of 70 ± 12% relative to the anaerobic treatment mean of 65 ± 19%. However, the percentage desorbed under aerobic conditions is higher for the low salinity and lower for the high salinity. This difference is likely due to the high variability in the data. As discussed previously, under anaerobic conditions there is increased competition from the reduced forms of Fe and Mn, as these forms (Fe^{2+} and Mn^{2+}) are more soluble. At higher salinities, where there is more competition from other cations, further competition from the reduced forms of Fe and Mn may contribute to an increased percentage of desorption.

6.3.2 Implications for the MUS

The data from this sorption study can be applied to other studies of the MUS investigating the treatment of nitrogen, as the primary component of the wastewater nitrogen is NH_4^+ (Turriciano, 2005; Fontenot et al., 2006). A column laboratory study designed to investigate the nitrogen processes within the MUS (Chapters 4 and 5) used the same soil that was used in this study. Two L of synthetic wastewater was added every other day using NH_4^+ concentrations of 100 and 80 mg NH_4^+-N L^{-1} (concentrations based on NH_4^+ values observed in

field data). The NH_4^+ effluent concentrations from the column study were used to extrapolate a saturation time. Raw data from the column experiment (Figure 6.4) shows the columns were approaching saturation towards the end of 84 days. This data was fit to a saturation curve (Equation 6.4) to determine the number of days required to reach saturation.

$$N = \frac{N_i \times t}{K_N + t} \tag{6.4}$$

where N is the NH_4^+ concentration (mg L^{-1}), N_i is the mean influent NH_4^+ concentration (mg L^{-1}), t is the time (y), and K_N is the constant (y). Fitting the raw data to a saturation curve yielded an approximate time to saturation for each column treatment (Figure 6.5). The columns were estimated to reach saturation, to within a few mg NH_4^+-N L^{-1} of the mean influent NH_4^+ concentration, between 108 to 246 days (Table 6.6).

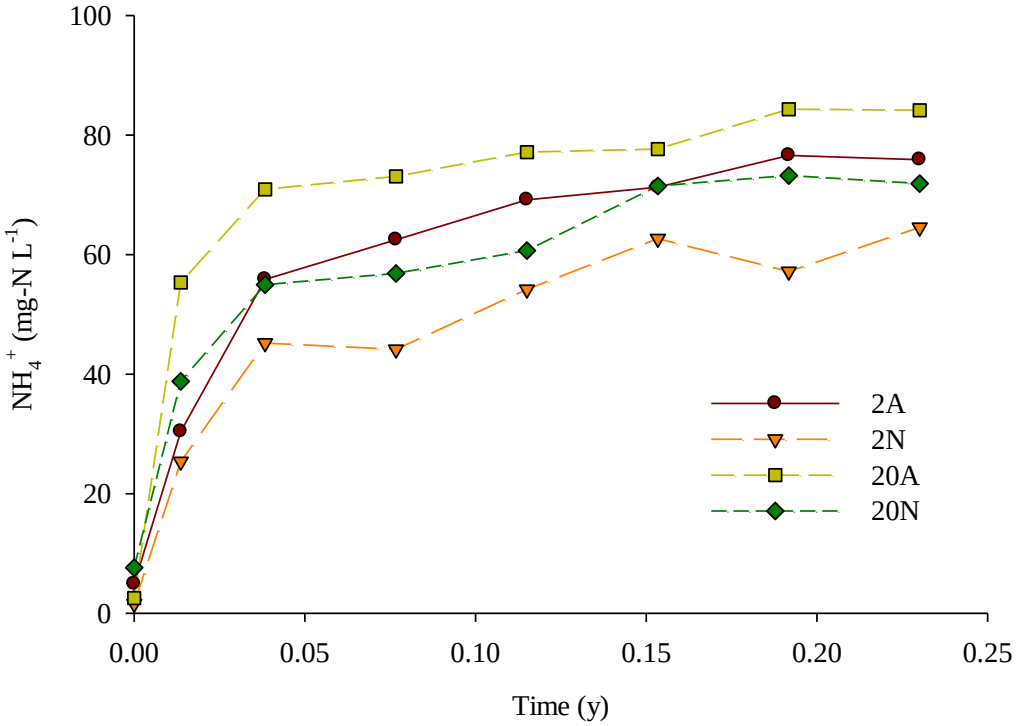


Figure 6.4 Raw column effluent NH_4^+ data by time. Treatments are abbreviated as follows for all figures: 2‰ (2), 20‰ (20), 100 mg NH_4^+-N L^{-1} (A), and 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N).

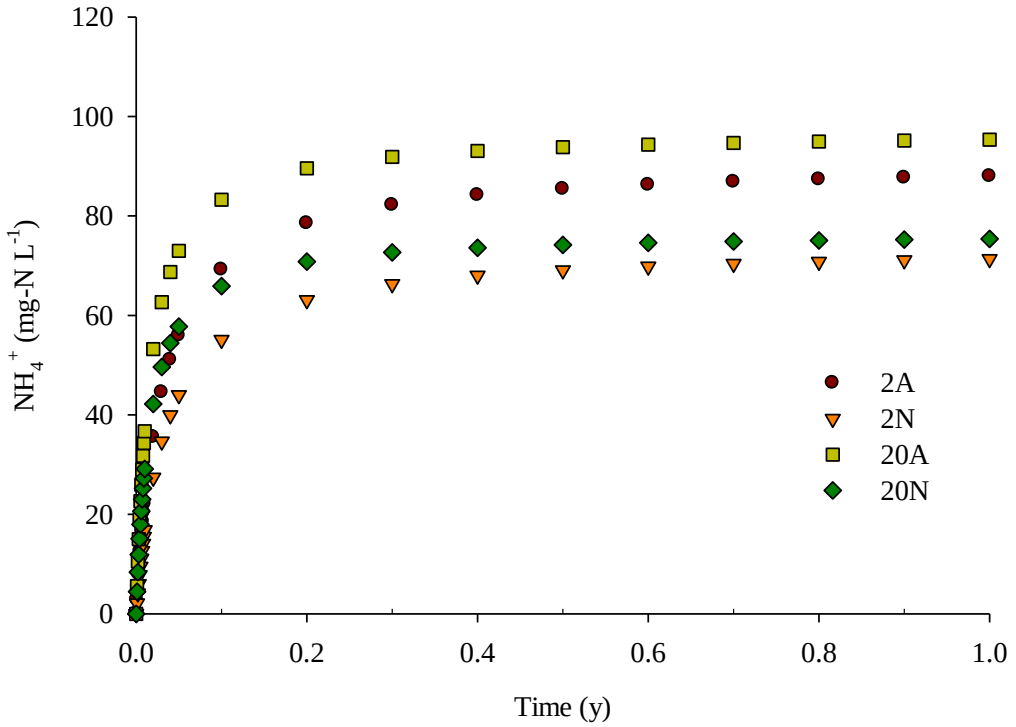


Figure 6.5 Extrapolated NH_4^+ data by time. Treatments are abbreviated as follows for all figures: 2‰ (2), 20‰ (20), 100 mg NH_4^+-N L^{-1} (A), and 80 NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (N).

The maximum sorption coefficient (β), estimated from the sorption Langmuir isotherms in this study, can be used to predict the length of time required to reach NH_4^+ saturation using equation 6.5.

$$L = \frac{\beta}{N_i \times Q} \times BD \times V \times 1000 \qquad (6.5)$$

where L is the longevity (d), β is the maximum sorption coefficient (mg NH_4^+-N kg $soil^{-1}$), N_i is the influent NH_4^+ concentration (mg NH_4^+-N L^{-1}), Q is the flowrate (L d^{-1}), BD is the bulk density (g soil cm^{-3}), V is the volume (m^3), and 1000 is a conversion factor. Using the estimated β's for the anaerobic treatment (Table 6.3), as conditions within the columns were anaerobic, and the known values in Table 6.7, the longevity equation estimated the saturation time for the column study to range from 26 – 96 days (Table 6.6). The predicted saturation times using the maximum sorption coefficient were less than saturation times found in the column study.

Table 6.6 Length of time (in days) required to reach NH_4^+ saturation in study columns and predicted longevity using N_i within a few mg NH_4^+-N L^{-1}.

NH_4^+ concentration	N_i	Saturation time	Predicted Longevity
2‰ at 100 mg L^{-1}	90.7	246	78
20‰ at 100 mg L^{-1}	96.9	139	26
2‰ at 80 mg L^{-1}	73.8	216	96
20‰ at 80 mg L^{-1}	76.6	108	33

Table 6.7 Values used for column longevity calculation.

Parameter	NH_4^+ treatment	Treatment	
		2‰ anaerobic	20‰ anaerobic
N_i (mg NH_4^+ L^{-1})	100 mg L^{-1}	90.7	96.9
	80 mg L^{-1}	73.8	76.6
Q (L d^{-1})		1	1
BD (g soil cm^{-3})		1.15	1.15
V (m^3)		0.015	0.015

However, if the mean of N_i is considered within one standard deviation and saturation times and longevity are recalculated using these values the discrepancy between saturation time and predicted longevity is not as great (Table 6.8). Thus, a prediction for field longevity of the MUS using the longevity equation and maximum sorption coefficient can be made. A direct comparison of sorption and desorption values obtained in this laboratory study cannot be applied directly to field conditions, but can still provide a rough estimation of saturation time. Cores obtained from a MUS field site found the bulk density ranged from 0.22 – 1.27 g cm^{-3} from the surface to a depth of 4.7 m. A weighted average of the bulk density gave a value of 0.605 g cm^{-3}. Though the exact treatment area is not known, as there are no boundaries within a field setting, an estimated treatment area of 300 m^3 was used. Field studies have found the mean influent NH_4^+ concentration to be 98 mg NH_4^+-N L^{-1}. Hydraulic loading rates at the Bayou Segnette field site ranged from 224 – 2,016 L d^{-1}. Based on these values, estimates of the length of time required to reach saturation in the field were found to range from 1.01 – 13.68 years at 2‰ and 0.33 – 3.22 years for 20‰ (Table 6.9). Using the percentage of NH_4^+ desorbed, a new

Table 6.8 Length of time (in days) required to reach NH_4^+ saturation in study columns and predicted longevity using N_i within one standard deviation.

NH_4^+ concentration	N_i	Saturation time	Predicted Longevity
2‰ at 100 mg L^{-1}	83.2	126	85
20‰ at 100 mg L^{-1}	89.8	76	28
2‰ at 80 mg L^{-1}	66.3	110	107
20‰ at 80 mg L^{-1}	69.5	58	36

Table 6.9 Estimated longevity of field system in years for a treatment area of 300 m^3.

Oxygen status	Salinity (‰)	q (mg NH_4^+-N kg $soil^{-1}$)	Ammonium saturation time (y) at various nitrogen loading rates (g NH_4^+-N day^{-1})			
			22	39	99	198
Aerobic	2	604	13.68	7.80	3.04	1.52
	20	132	2.99	1.70	0.66	0.33
Anaerobic	2	402	9.11	5.19	2.02	1.01
	20	142	3.22	1.83	0.71	0.36

estimate of saturation time can be obtained by applying the mean percent desorbed to the NH_4^+ loaded. This changes the estimated saturation times to 1.76 – 19.99 years at 2‰ and 0.48 – 4.30 years for 20‰ (Table 6.10).

As saturation is reached for the soils nearest the injection point, the NH_4^+ within the wastewater will move out and saturate sorption sites at a point further away. Two field studies of the MUS in an intermediate and saltwater marsh had wastewater plumes of ~2 and 20‰, respectively. After a year of system operation, neither field site saw saturation. The mean

Table 6.10 Estimated longevity of field system in years for a treatment area of 300 m^3 including desorption.

Oxygen status	Salinity (‰)	q (mg NH_4^+-N kg $soil^{-1}$)	Ammonium saturation time (y) at various nitrogen loading rates (g NH_4^+-N day^{-1})			
			22	39	99	198
Aerobic	2	604	19.99	11.39	4.44	2.22
	20	132	4.20	2.39	0.93	0.47
Anaerobic	2	402	15.85	9.03	3.52	1.76
	20	142	4.30	2.45	0.96	0.48

nitrogen loading rates observed at these sites were 21 and 31 g NH_4^+-N day^{-1} for the intermediate and saltwater marshes, respectively. A comparison of the estimated anaerobic saturation times to field data (as anaerobic conditions were found in the field) shows the estimated times (between 2.5 – 16 years) are within the range of field data. However, this comparison does not speak to the longevity of the MUS, but as the treatment area is unknown, the length of time it would take to reach saturation is also unknown. Additionally, adjacent camps utilizing the MUS would decrease the amount of time in which saturation would be reached due to overlapping wastewater plumes and an increase in NH_4^+ loading within the subsurface.

6.4 Conclusion

The Langmuir isotherm provided a more adequate fit over linear and Freundlich isotherms for NH_4^+ sorption in this study. Increases in salinity led to a significant decrease in the amount of NH_4^+ sorbed but only slightly increased the amount of NH_4^+ desorbed. Oxygen level (aerobic or anaerobic) had little effect on either sorption or desorption. Soil properties, native groundwater salinity, and available treatment area should be considered when installing systems in close proximity. Saturation of soil sorption sites for a laboratory column study took 110 – 126 days under 2‰ and 58 – 76 days for 20‰. Prediction of saturation times using the maximum sorption coefficient from the Langmuir sorption isotherm was found to be between 85 – 107 days under 2‰ and 28 – 36 days for 20‰. Using the same prediction equation the MUS, within a 300 m^3 treatment area, was predicted to last between 0.33 – 13.68 years depending upon the salinity. However, an exact treatment area is not known and, therefore, the sustainability of long-term use of the MUS for the treatment of NH_4^+ is also not known.

CHAPTER 7: GLOBAL CONCLUSIONS AND FUTURE RESEARCH

7.1 Background Review

Coastal wetland environments in Louisiana and Mississippi are typified by high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater. The marshland upwelling system (MUS) was designed to utilize the prevalent natural wetlands in coastal areas for effective and relatively (as compared to current publicly available technologies) cheaper wastewater treatment for coastal camp dwellings. As such, the MUS is unique among wetland wastewater technologies. The studies presented herein considered the effectiveness of the MUS for the treatment of organic matter and nitrogen found in domestic wastewater.

The main questions of these studies were to determine:

1) What is the effectiveness of the MUS in removing organic matter from the wastewater as measured by five-day carbonaceous biochemical oxygen demand ($CBOD_5$)?
2) What nitrogen transformations are occurring, at what rate, and what is the fate of the forms generated within the MUS?
3) Will an oxidizing treatment in the primary collection tank convert substantial amounts of ammonium to nitrate prior to injection?
4) Can denitrification of wastewater nitrate be significantly enhanced and, thus, lead to removal of nitrogen from the system?
5) Does a more saline environment significantly impact the treatment of $CBOD_5$ and the rate of nitrogen removal?

7.2 Global Conclusions

The MUS was found to be highly effective in the removal of organic matter (as measured by $CBOD_5$) and in the removal of dissolved organic carbon (DOC). In two field studies, $CBOD_5$ was found to have removal efficiencies of 95 and 99% for a saltwater (native salinity of ~ 30‰)

and intermediate salinity (~10‰) marsh, respectively. A laboratory column study found a 90% removal efficiency of DOC. There was some indication that a high salinity negatively affected the treatment of carbon during the column study. The high salinity (20‰) treatment had a significantly higher DOC effluent near the beginning of the study. The difference in effluent DOC, however, decreased with increased time and became insignificant by day 56, suggesting that there may be a lag in the effective treatment of carbon in high salinity marshes. A lag time in treatment efficiency was also suggested by field studies, as treatment efficiency increased with each sequential study. A lag time would be expected if the native microbial population required acclimation to the introduction of wastewater.

Introduction of oxygen into a wastewater solution in a laboratory study was found to bring about nitrification and occurred more rapidly under a C:N ratio of 2:1 relative to a ratio of 4:1. Nitrification began to occur after a lag time of approximately two weeks and had a mean maximum conversion rate of 20%. This was surprising as heterotrophic microorganisms are expected to out compete nitrifying bacteria for resources (i.e., carbon and oxygen) and the maximum amount of conversion was not anticipated to reach 20% (Metcalf and Eddy, 2003).

Injected NH_4^+ was found to be primarily treated by sorption to soil particles during a laboratory column study. As soil sorption sites became saturated, more NH_4^+ was released into the column wastewater effluent. After 84 days, NH_4^+ removal efficiency was only found to be 20%; however, the treatment area of the columns was a confined, limited volume. Field systems would have a higher removal efficiency, as the treatment area is unbounded. The length of time to saturation of soil sorption sites would be dependent upon the type of soil present and the volume of the treatment area.

Nitrate injected had a removal efficiency of > 99%. Measures of denitrification enzyme activity (DEA), which ranged from 0.001 to 22.6 mg N_2O-N kg $soil^{-1}$ h^{-1}, found that

approximately 41% of wastewater NO_3^- was removed via denitrification within a few cm of the injection site. The removal of the other portion of NO_3^- is thought to be by anammox or dissimilatory nitrate reduction to ammonia as conditions within the column would also support these microbial processes. Total carbon and organic matter were also positively correlated with increases in DEA; therefore, influx of wastewater into the MUS subsurface would increase the rates of denitrification and, subsequently, increase the removal of nitrogen via N_2 gas.

Salinity significantly affected the sorption of NH_4^+, with lower salinity (2‰) soils having a higher sorption capacity for NH_4^+ relative to higher salinity (20‰) soils. Thus, the length of time it would take the treatment area to saturate with NH_4^+ would be expected to be longer in marshes with a lower native groundwater salinity. A high salinity (20‰) was also found to have a negative impact on microbial activity as measured by DEA and potentially mineralizable nitrogen (PMN) rates. The negative impact of a higher salinity on microbial processes, specifically DEA, could potentially reduce the treatment effectiveness of systems installed in marshes of higher salinities.

7.3 Recommendations for Future Research

Overall, the MUS has shown to be very effective for treatment of carbon and nitrate nitrogen. However, certain changes may lead to improved treatment and increased longevity of field systems and are recommended for future studies of the MUS. Consideration of a lag time by decreasing the initial wastewater load to allow the microbial population time to acclimate to the changing conditions introduced by wastewater injection may help to improve initial carbon treatment. Modification of the primary collection/distribution tank to aerate wastewater (i.e., increase oxygen) should lead to an increase in nitrification and, thus, reduce the NH_4^+ load to the subsurface. Field studies should be performed to determine the best aeration rate and design to achieve maximum NH_4^+ conversion to NO_3^-. This is especially important for systems installed

in higher salinity marshes, as less injected NH_4^+ will lead to an increase in longevity since sorption capacity for ammonium is lower at higher salinities. Overlapping of wastewater plumes from adjacent systems may decrease longevity of the MUS for treatment of NH_4^+ as it is primarily treated by sorption and an increased NH_4^+ load will more quickly decrease the number of sorption sites available. Future studies should look at the distance between systems and the effect of overlapping plumes. With modifications, the MUS stands to become the first, long-term, effective treatment system for treatment of coastal dwelling wastewater which currently plays a role in the reduction of water quality in the coastal zone.

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APPENDIX A: TECHNICAL NOTE: OPTIMIZATION OF WASTEWATER NITRIFICATION IN THE MARSHLAND UPWELLING SYSTEM

A.1. Introduction

The natural properties wetlands possess make them ideal for wastewater treatment as most wetlands are sinks for chemicals, particularly nutrients. Treatment wetlands have been used for a variety of applications, including domestic wastewater, mine drainage, nonpoint source pollution, stormwater runoff, landfill leachate, and confined livestock operations (Gopal, 1999; Sun et al., 1999; Cardoch et al., 2000; Mitsch and Gosselink, 2000; Revitt et al., 2001; Howarth et al., 2002; Al-Omari and Fayyad, 2003; Ansola et al., 2003; Mantovi et al., 2003; Poach et al., 2003; Steinmann et al., 2003). In the coastal wetland environments of Louisiana, the majority of current onsite wastewater treatment and disposal systems technologies, such as septic systems, do not work well under conditions typifying the area; such as high water tables, poor hydraulic soil conditions, anaerobic soils, and saline groundwater. To address this problem and utilize the prevalent natural wetlands along coastal areas, the marshland upwelling system (MUS) was developed as an alternative wastewater treatment system (Stremlau, 1994; Watson Jr. and Rusch, 2001, 2002; Richardson et al., 2004; Richardson and Rusch, 2005; Fontenot et al., 2006; Evans and Rusch, 2007a).

Black and gray wastewater collected from coastal dwellings is held in a collection/distribution tank. Settled wastewater is intermittently injected into the marsh subsurface by means of an injection well. A number of natural processes treat the wastewater as the plume moves through varying zones of oxidation-reduction capacities. These processes include physical filtration, die off (of microorganisms), nutrient adsorption by mineral and organic soil solids, plant uptake, and microbial decomposition. Anaerobic and anoxic microbial processes in the subsurface can lead to transformations of carbon and nitrogen to gaseous forms,

which are enhanced by coupling with aerobic processes occurring at the soil surface and rhizosphere. Wastewater in the MUS has a high ammonium (NH_4^+) component (98% of total nitrogen injected). In addition, very little dissolved oxygen (DO) is present and, therefore, little nitrification can occur to convert NH_4^+ to nitrate (NO_3^-) before injection. Thus, the majority of nitrogen injected into the anaerobic subsurface is in the form of NH_4^+. If more oxygen were introduced into the collection tank, nitrification could potentially occur and the subsequent injection of some NO_3^- into the anaerobic subsurface could lead to denitrification and loss of nitrogen from the system. Therefore, the objectives of this study were to: 1) determine the potential to convert some NH_4^+ to NO_3^- via air injection within the collection tank, 2) determine the optimal flow rate of air, and 3) determine the maximum conversion of NH_4^+ to NO_3^-.

A.2. Material and Methods

Primarily treated wastewater was obtained from the Baton Rouge City Parish Wastewater Central Treatment Plant, Baton Rouge, Louisiana. Three gallons (11.35 L) of wastewater underwent two treatments: two air flows (6 and 12 SCFH, standard cubic feet per hour) and two C:N ratios (2:1 and 4:1) for a total of four treatment combinations, which were run in triplicate. The wastewater was spiked to a final concentration of approximately 15 mg L^{-1} PO_4-P, 50 (2:1 C:N) or 100 (4:1) mg L^{-1} NH_4-N, and 100 (2:1) or 400 (4:1) mg L^{-1} of C from a 50/50 mixture of dextrose and glutamic acid. The initial pH was adjusted to 7.5 with $NaHCO_3$. The buckets were placed in a water bath to keep the temperature stable at 24°C. The experiment was repeated a second time using both C:N ratios but only the 6 SCFH treatment. Each treatment combination was triplicated and spiking concentrations remained the same. The experiment was repeated because the high pH in the first trial resulted in a loss of N. The initial pH was adjusted to 7.5 as in the first trial; however, the pH was adjusted throughout the study with diluted HCl to maintain

a pH below 8. The second trial was also inoculated with nitrifying bacteria obtained from fish pond backwash.

Initial total ammonium nitrogen (TAN), total organic carbon (TOC), dissolved organic carbon (DOC), soluble reactive phosphorus (SRP), and metals were measured from the wastewater. Sampling occurred every six hours for the first 48 hours and was then reduced to every 12 hours until day five. After one week, sampling was reduced to every 48 hrs until day fourteen. Dissolved oxygen, pH, temperature, TAN (method 4500-NH_3 D, filtered (0.45 μm GF/C glass fiber filters) and unfiltered), TOC and DOC (Shimadzu TOC-CHSN, Columbia, MD), total nitrogen (TN, Shimadzu TOC-CHSN), nitrite (NO_2^-, 4500-NO^-_2 B), and NO_3^- (4500-NO^-_3 E) were measured for every sampling event (APHA, 1998). Total Kjeldahl nitrogen (TKN, 4500-N_{org} C and 4500-NH_3 D) and SRP (4500-P E) were measured at the beginning and end (APHA, 1998).

SAS® software (2009) and SigmaPlot® software (2002) were used to analyze data. All statistical tests were performed at a significance level of $\alpha = 0.05$. The treatment means for organic carbon were regressed against time using a second-order decay equation (Kadlec and Knight, 1996):

$$C = \frac{1}{\frac{1}{C_o} + tx} \quad (1)$$

where C_o is the initial concentration (mg L^{-1}), t is the time (h), and x is the decay constant (h^{-1}). Decay constants were compared statistically by fitting the decay equation to each set of data. Taking the estimated decay constants from each data set, a two-way or one-way ANOVA was used to determine significant treatment effects of air flow rate and C:N ratios on the decay constants.

A.3. Results and Discussion

A total of 216 samples were taken during the first trial. Total organic carbon and DOC showed rapid drops within the first 24 h (Figure A.1, only TOC shown). By the end of 48 h, TOC and DOC had begun to reach a steady state. Decay constants (Table A.1) showed no significant difference between flowrate treatments. However, there was a significant difference in the decay constant between the C:N ratios, with the 2:1 ratio being higher ($p < 0.001$). There were no differences seen in the DO level between flowrate treatments (Figure A.2).

Ammonium showed a general decrease throughout the study (Figure A.3, only filtered shown). Total nitrogen also showed this trend, indicating nitrogen was lost from the system. The pH initially started at 7.5, but quickly rose to between 8.5 and 9.5 (Figure A.2). The range of pH from 8.5 to 10 dramatically increase the rate of volatilization (Reddy and Patrick, 1984),

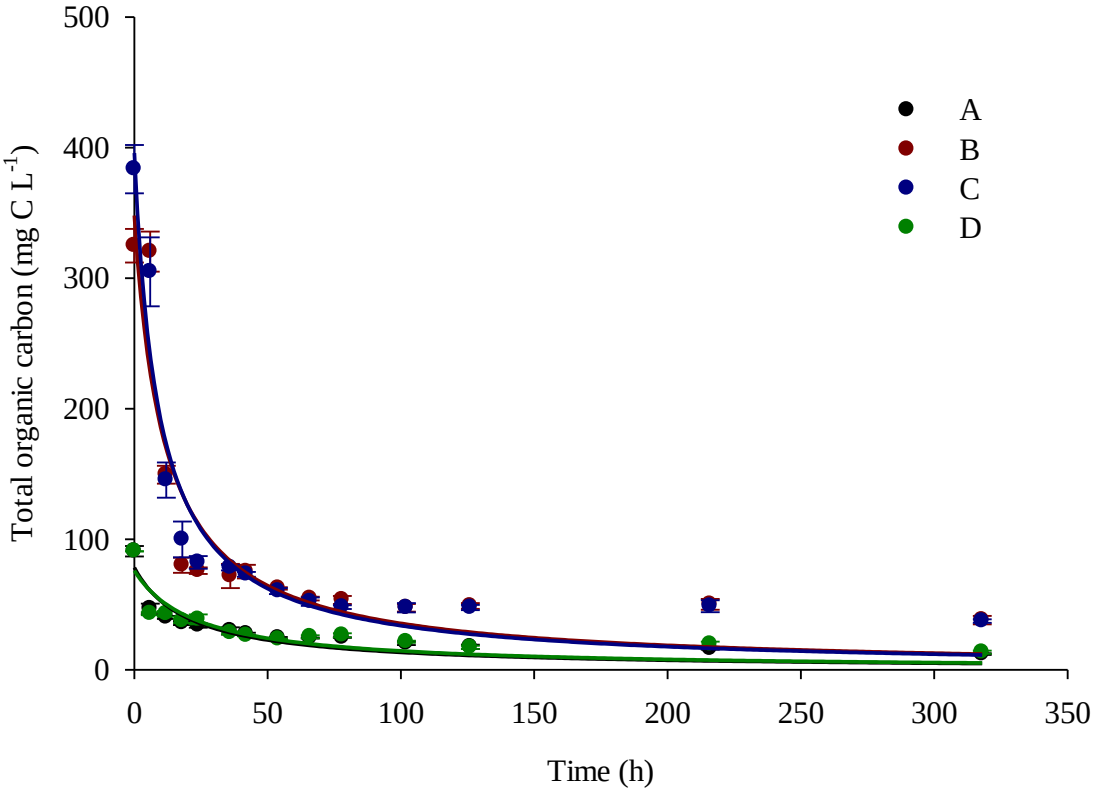

Figure A.1 Total organic carbon by time for the first trial. Dots and error bars represent data. Lines show fitted second order equation. Treatments A – D can be found in Table A.1.

Table A.1 Treatment names, TOC decay constants, and r^2 values for the first trial.

Treatment Name	C:N ratio	Flowrate SCFH	Decay Constant (h^{-1})	r^2 (%)
A	2:1	6	0.0006	0.834
B	4:1	6	0.0003	0.865
C	4:1	12	0.0003	0.934
D	2:1	12	0.0006	0.789

which changes the soluble form, NH_4^+, to the gaseous and more volatile form, NH_3. The pH increase likely lead to a loss of NH_4^+ from the system. The loss of NH_4^+ is seen in Figure A.4 where the mass balance (green line) of nitrogen forms decreases substantially from the initial nitrogen amount. If no nitrogen was lost from the system, the line should be steady throughout the entire study and only the amounts of different nitrogen forms should change as nitrification begins to consume NH_4^+ and produce NO_2^- and NO_3^-. Nitrite did not start to increase until after hour 100 and reached close to 40 and 5 mg N L^{-1} by the end of the two weeks for the 2:1 and 4:1 C:N ratios, respectively (data not shown). Again, there were no differences seen between

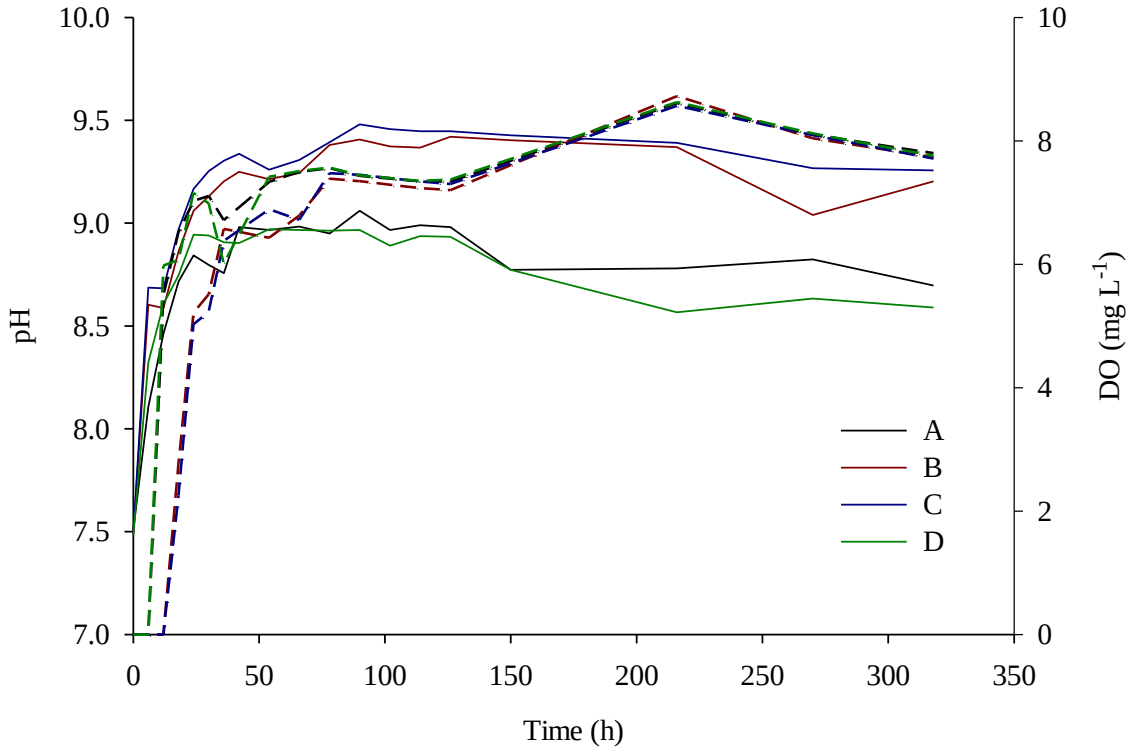

Figure A.2 The pH and DO from the first trial. Dashed lines shown represent DO.

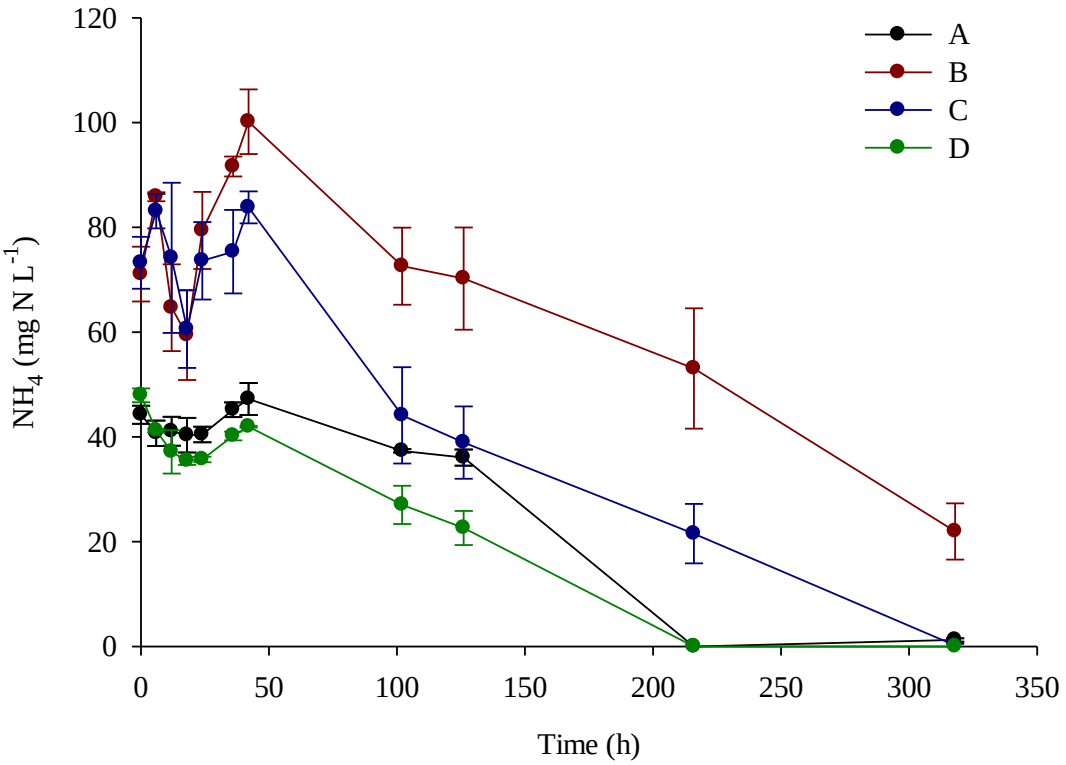

Figure A.3 Filtered NH_4^+ by time from the first trial.

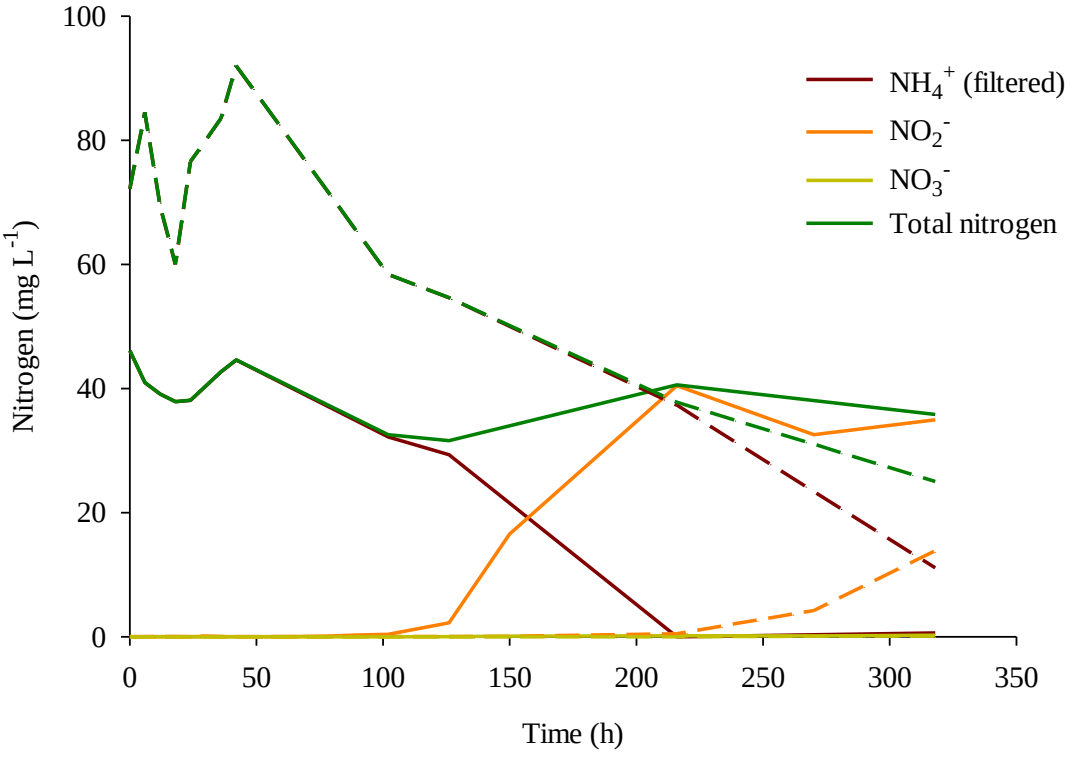

Figure A.4 Comparison of nitrogen components by time with total nitrogen mass balance for the first trial. Solid lines are mean of A+D and dashed lines are mean of B+C.

flowrates. Nitrate had barely begun to increase by the end of the study, and never reached above 0.3 mg N L^{-1} for any treatment. The delay in nitrification likely occurred because nitrifying organisms grow more slowly than heterotrophic microorganisms, and thus heterotrophs dominated and consumed the majority of the carbon before nitrifiers grew enough to utilize the carbon (Metcalf and Eddy, 2003). In addition, the substrate used most likely did not have an established community of nitrifying bacteria, thus adding to the time it took for nitrification to begin. The lag time present between the first and second steps of nitrification could potentially be explained by the high pH, as the bacteria responsible for the second step, *Nitrobacter*, are highly sensitive to changes in pH, particularly high pH values (Reddy and DeLaune, 2008).

The experiment was run a second time using 6 SCFH with only the 4:1 and 2:1 C:N ratios as treatments, as there was no difference seen in treatment between the flowrate treatment. The second trial took into account the rising pH and lack of an established nitrifying community and corrected for these. The pH was adjusted to below 8 at every sampling event and backwash from a fish pond filter was added at the beginning of the trial in an attempt to introduce a more established nitrifying community. In the second trial, 114 samples were collected and analyzed. The same trend was seen in the carbon data (Table A.2, Figure A.5) with the decay constants still being significantly different ($p < 0.001$) between the C:N ratio treatments.

Only the first 48 h saw differences in DO between the two treatments (Figure A.6), after 48 h, little difference was seen between the DO in both treatments. Ammonium showed an initial decrease (Figure A.7, only filtered shown), but then rose to near steady state until hour

Table A.2 Treatment names, TOC decay constants, and r^2 values for the second trial.

Treatment Name	C:N ratio	Decay Constant (hr^{-1})	r^2 (%)
L	2:1	0.0009	0.905
H	4:1	0.0003	0.953

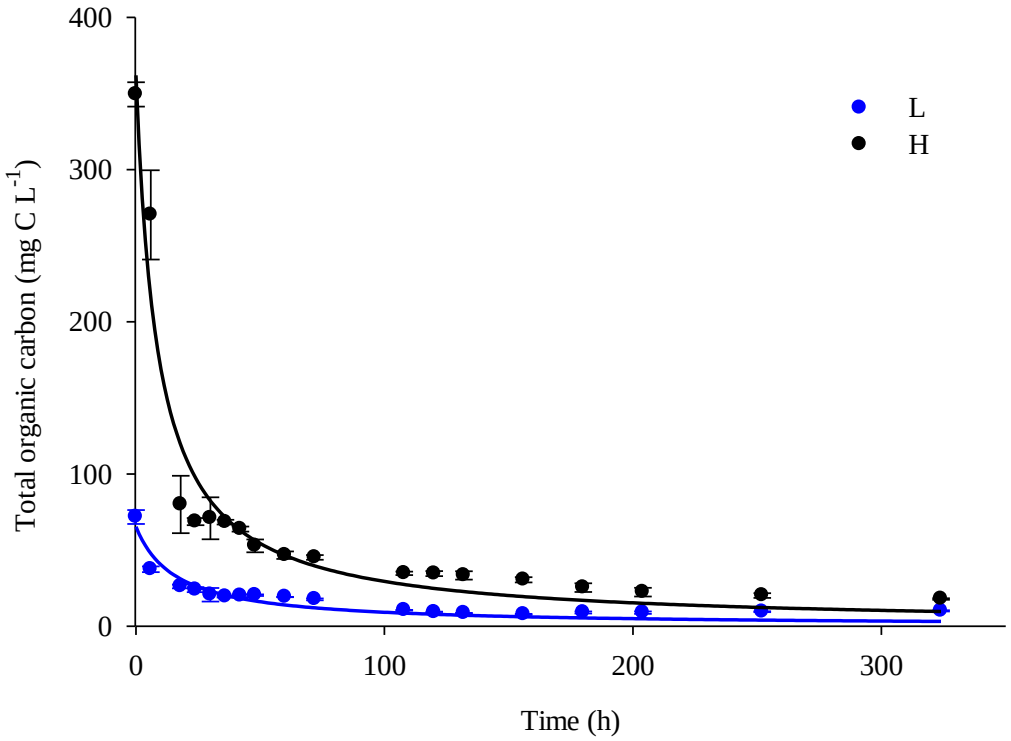

Figure A.5 Total organic carbon by time for the second trial. Dots and error bars represent data. Lines show fitted second order equation. Treatments L and H can be found in Table A.2.

120 (for L) and 200 (for H), when it began to decrease. Total nitrogen showed a similar trend in the beginning, with a decrease in nitrogen concentration in the first 24 h, but then nitrogen reached a steady state around 100 and 50 mg N L^{-1} for the H and L treatments, respectively. The pH initially started at 7.5, but quickly rose to approximately 8.5 as in the first trial (Figure A.6). The initial increase in the pH most likely explains the initial loss seen in the NH_4^+, as some NH_4^+ volatilized and was lost from the system (Figure A.8). However, pH did not continue to rise, but instead began to decrease, reaching a pH between 5.5 and 6.0 by the end of the study. The pH began to decrease substantially after hour 150. At this point, nitrification had begun to occur and the rate at which it occurred continued to increase, which explains the continued drop in pH. The first step in nitrification, ammonia oxidation, produces protons, thus decreasing the pH. However, if the pH continued to decrease, nitrification rates would start to taper off and possibly even decrease, as nitrification rates begin to decrease as the pH drops below 6.8 (Metcalf and

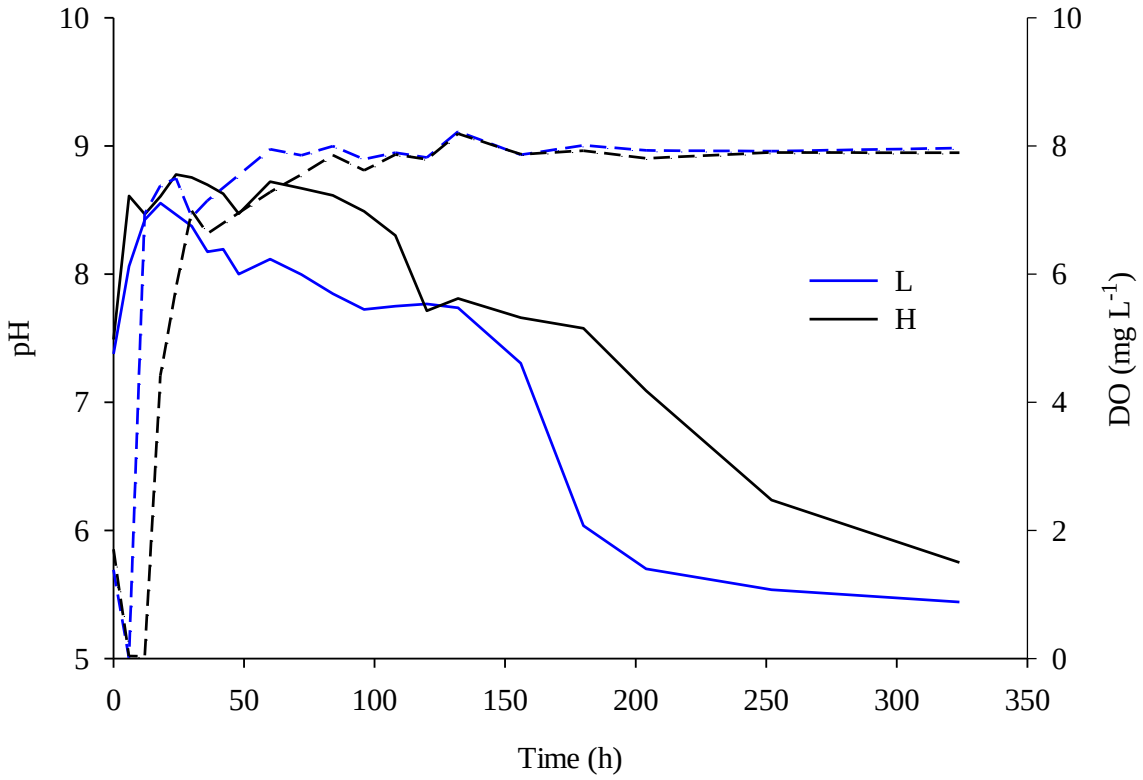

Figure A.6 The pH and DO from the second trial. Dashed lines represent DO.

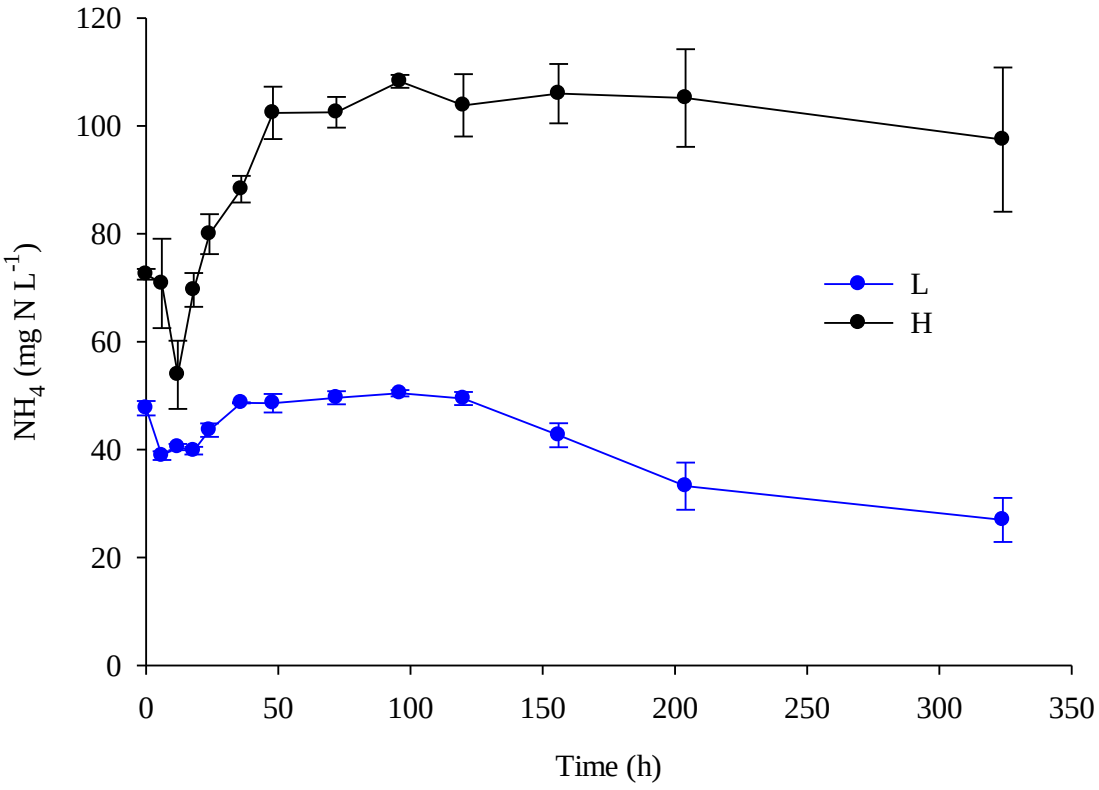

Figure A.7 Filtered NH_4^+ by time from the second trial.

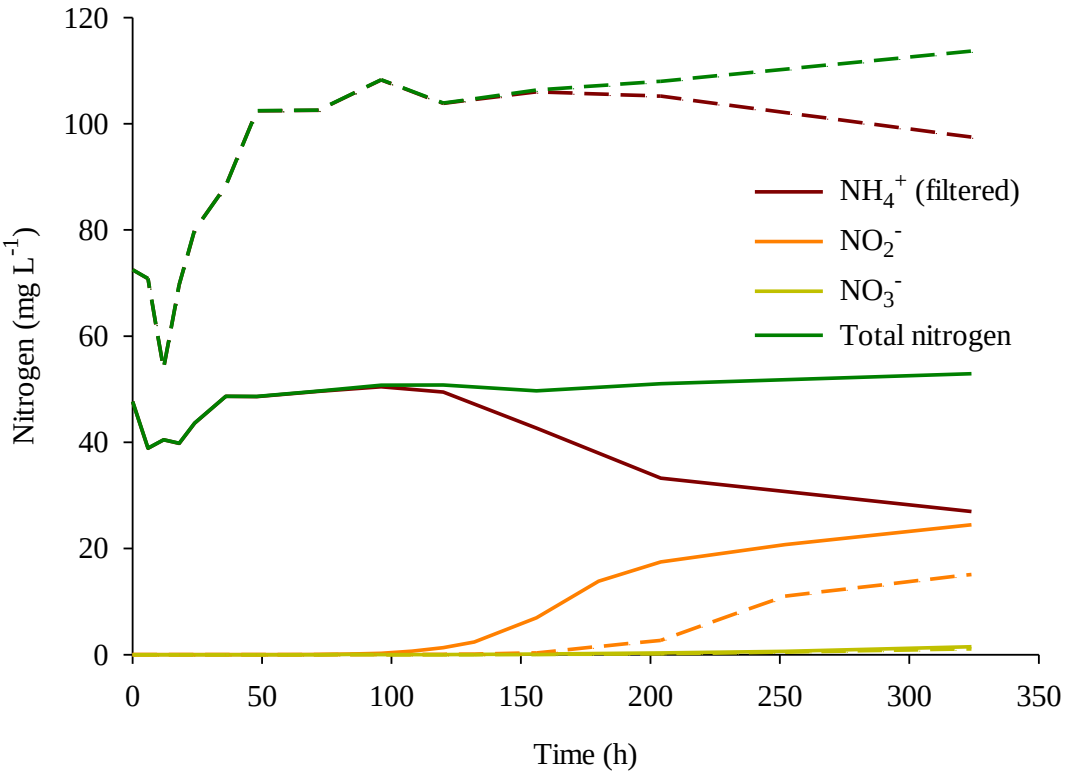

Figure A.8 Comparison of nitrogen components by time with total nitrogen mass balance for the second trial. Solid lines are L and dashed lines H.

Eddy, 2003; Reddy and DeLaune, 2008). Nitrite began to increase between hours 100 – 150 for the L treatment and between hours 150 – 200 for the H treatment and reached 15 to 25 mg NO_2^--N L^{-1} for H and L, respectively (Figure A.9). Nitrate began to increase around the same times as NO_2^-, but only reached 1.0 and 1.5 mg NO_3^--N L^{-1} by the end of the two weeks for H and L, respectively. The lag time between the first and second steps of nitrification is likely due to the pH, as the second step is more susceptible to changes in pH (Reddy and DeLaune, 2008).

A.4. Conclusion

This study clearly demonstrated the consumption of carbon in wastewater by heterotrophic bacteria and subsequent carbon utilization and nitrite/nitrate production by nitrifying bacteria (Metcalf and Eddy, 2003). Two air flowrates and C:N ratios were used to determine the influences of increased airflow and higher C:N ratios. The first trial in the study showed no significant differences in the carbon decay constant between air flowrates. However,

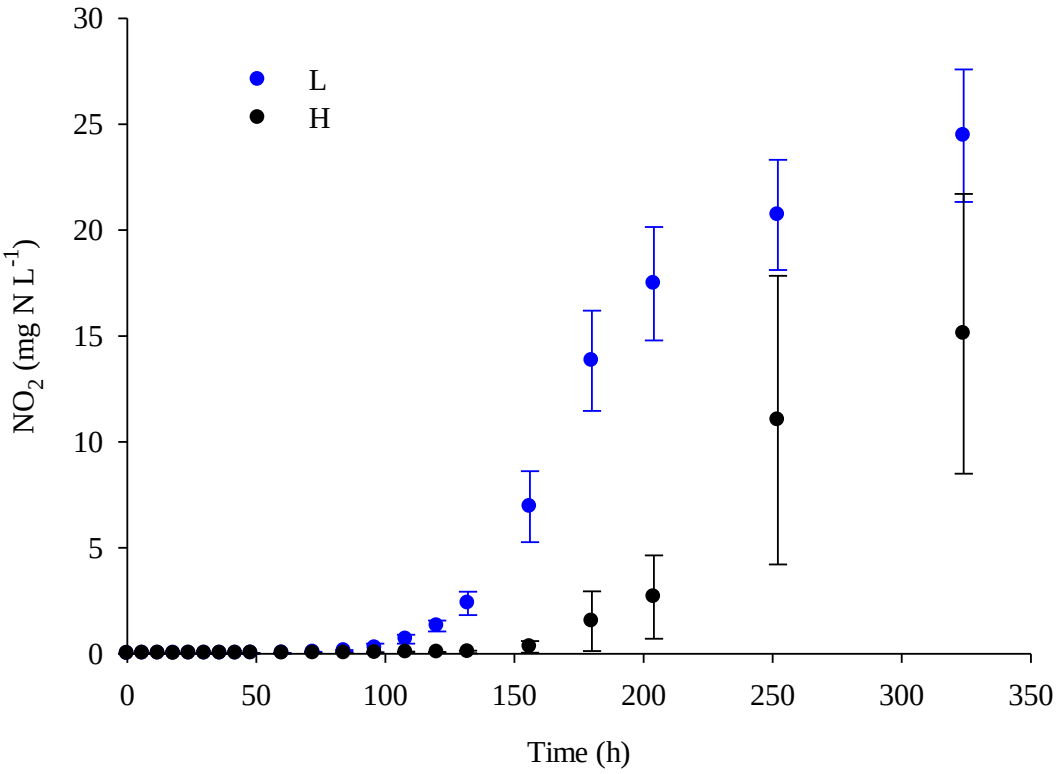

Figure A.9 Nitrite by time from the second trial.

both trials showed significant differences between C:N ratios, with the lower C:N ratio (2:1) having the higher decay constant. The study was designed to determine optimal conditions for the production of NO_2^-/NO_3^- before wastewater injection into an anaerobic environment and the maximum conversion of NH_4^+ to NO_2^-/NO_3^- for use in further trials. Based on the results of this study, the optimum C:N ratio for conversion is 2:1 over a 4:1 ratio. The amount of NO_2^-/NO_3^- produced from the 2:1 ratio is 50% of the total NH_4^+, while the 15% of the NH_4^+ was converted in the 4:1 ratio. Field studies have shown the C:N ratio to be closer to the 4:1 ratio (Turriciano, 2005; Fontenot et al., 2006); however, wastewater collection tanks would likely have a well-established nitrifying community present, which may increase the amount of NH_4^+ converted. Therefore, a reasonable estimate of the maximum amount of NH_4^+ conversion to NO_2^-/NO_3^- expected to occur, before injection into the anaerobic subsurface, is approximately 20% of the NH_4^+ concentration.

APPENDIX B: ADDITIONAL GRAPHS

B.1. Chapter 4 Graphs

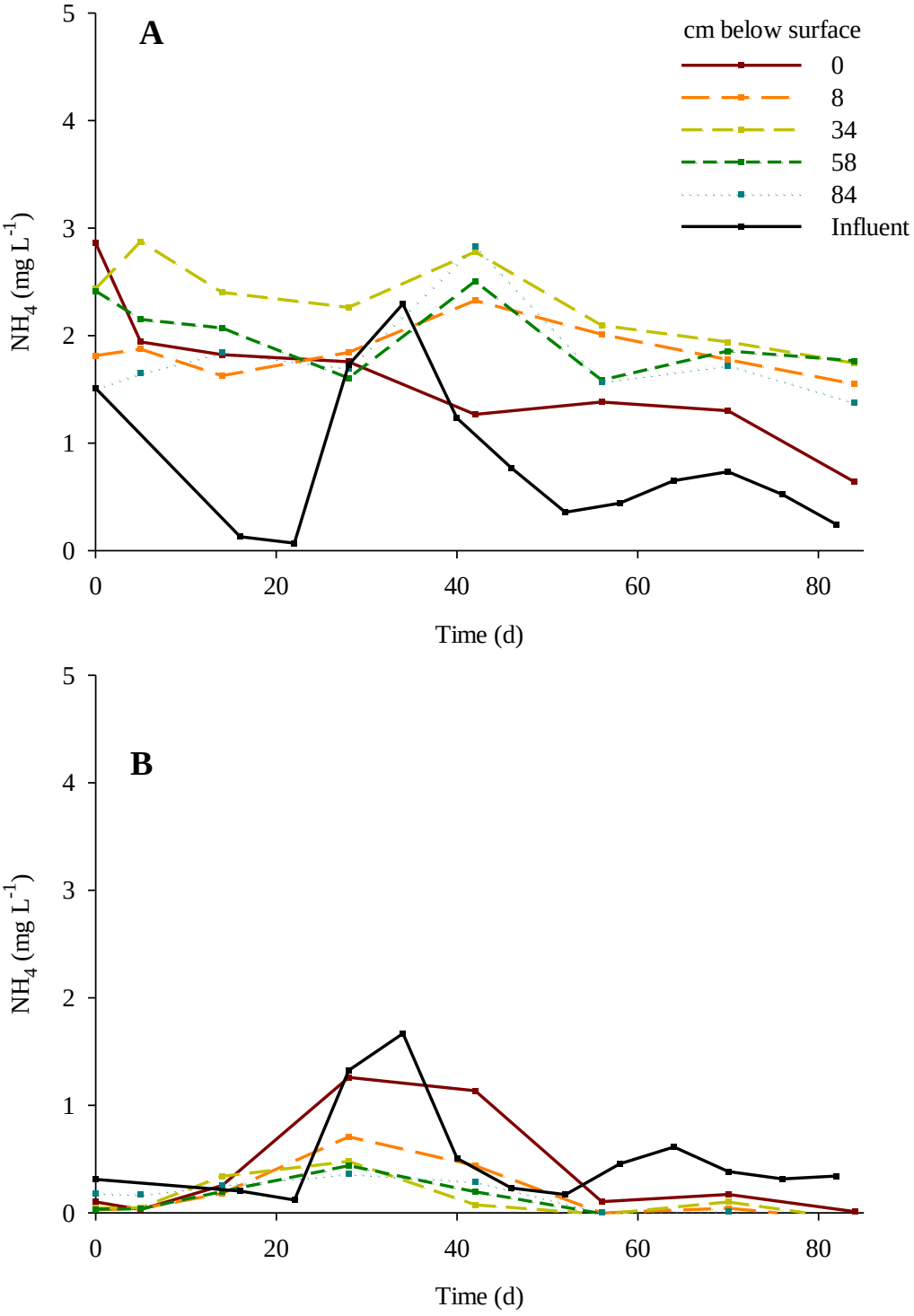

Figure B.1 Graph of NH_4^+ versus time for control columns 20‰, planted, no wastewater (A) and 2‰, planted, no wastewater (B). Lines represent the mean at each depth.

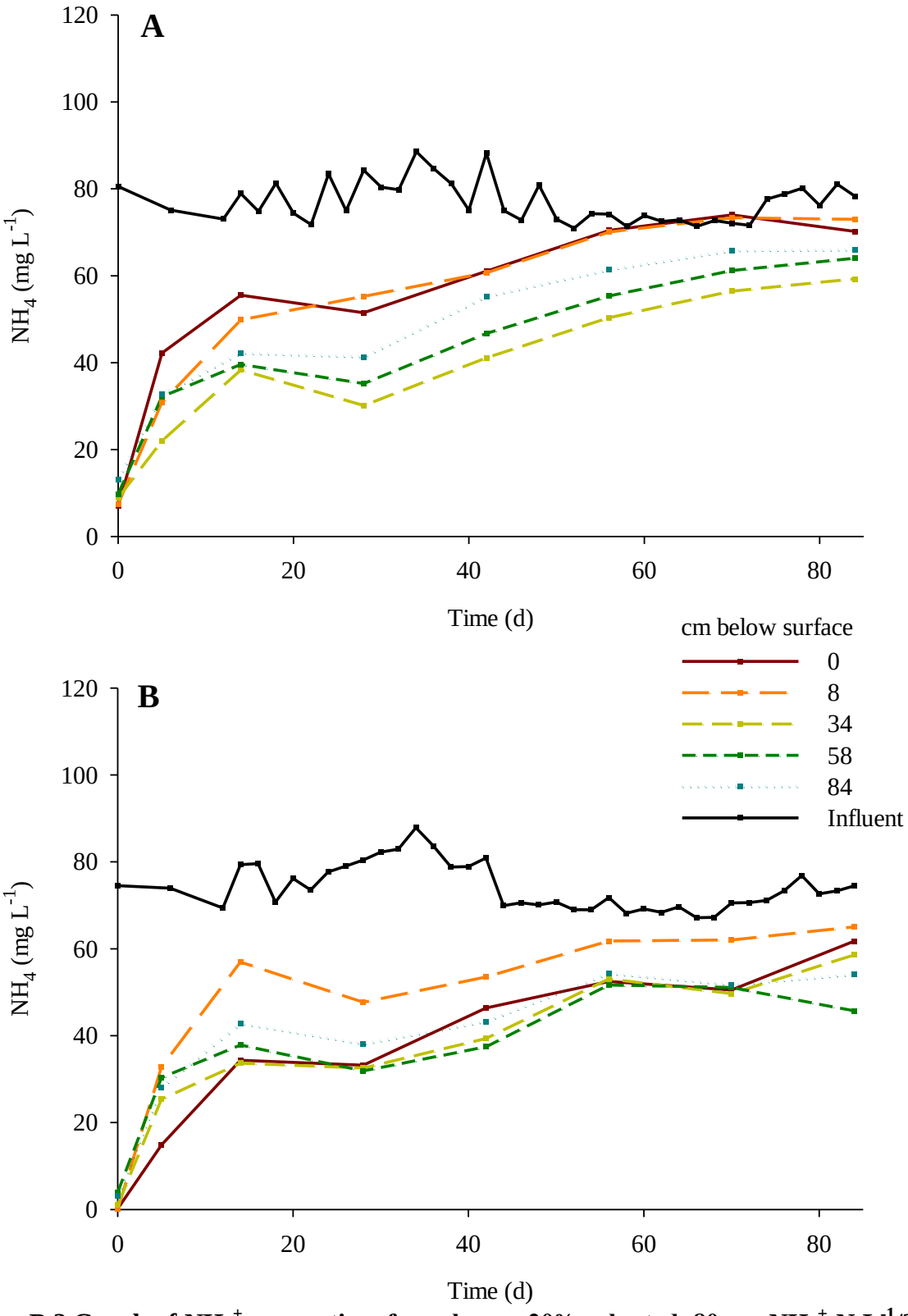

Figure B.2 Graph of NH_4^+ versus time for columns 20‰, planted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (A) and 2‰, planted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (B). Lines represent the mean at each depth.

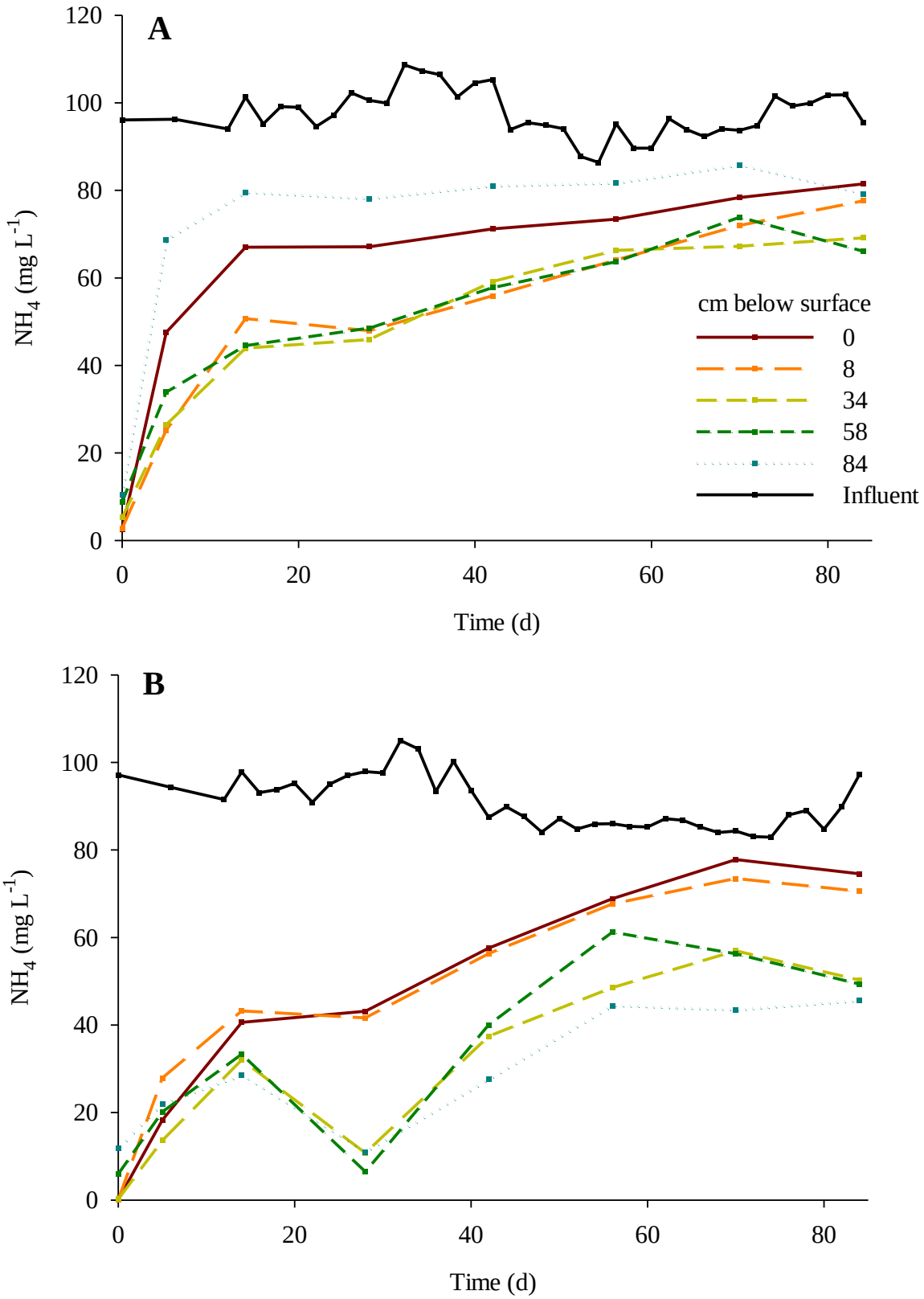

Figure B.3 Graph of NH_4^+ versus time for columns 20‰, planted, 100 mg NH_4^+-N L^{-1} (A) and 2‰, planted, 100 mg NH_4^+-N L^{-1} (B). Lines represent the mean at each depth.

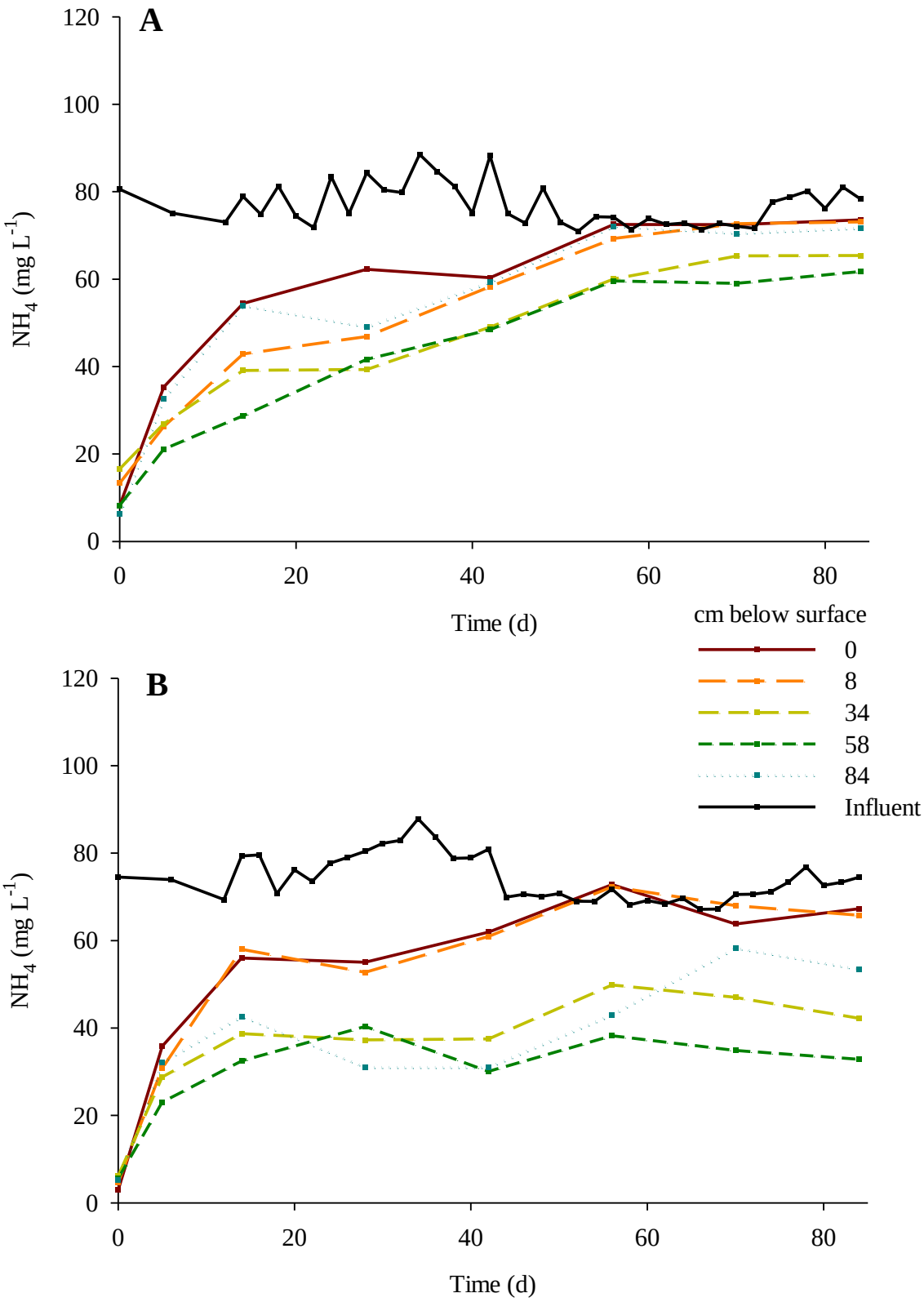

Figure B.4 Graph of NH_4^+ versus time for columns 20‰, unplanted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (A) and 2‰, unplanted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (B). Lines represent the mean at each depth.

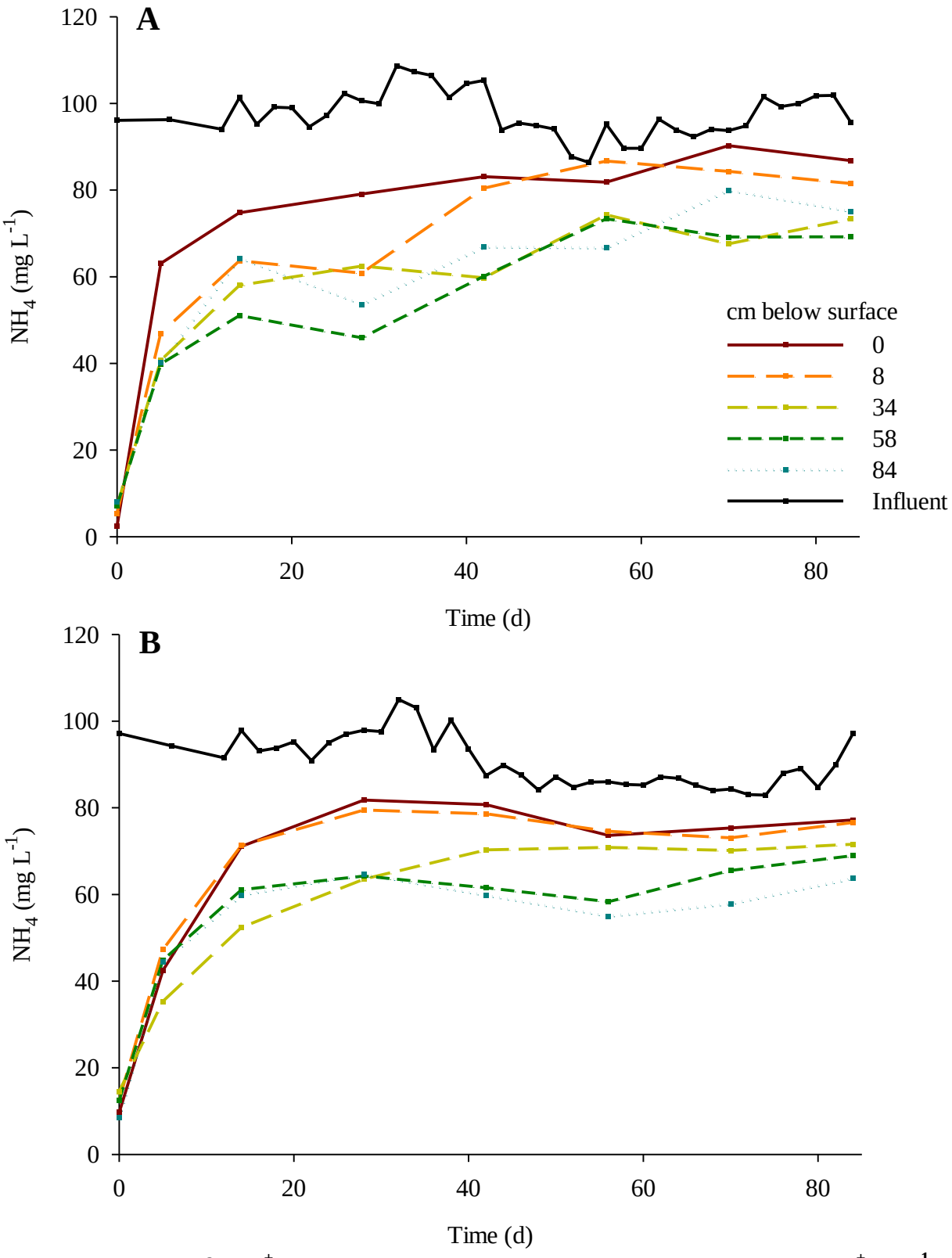

Figure B.5 Graph of NH_4^+ versus time for columns 20‰, unplanted, 100 mg NH_4^+-N L^{-1} (A) and 2‰, unplanted, 100 mg NH_4^+-N L^{-1} (B). Lines represent the mean at each depth.

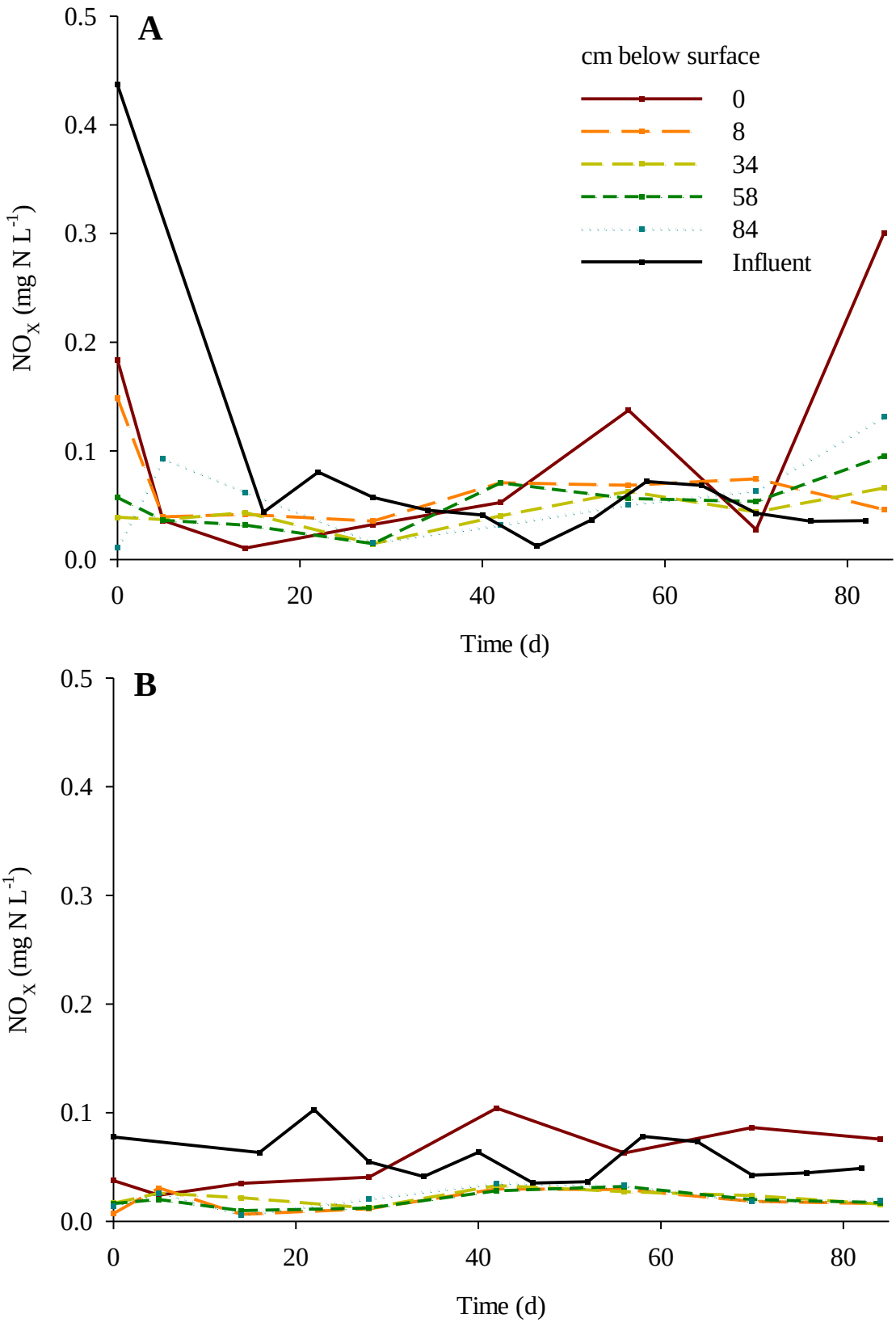

Figure B.6 Graph of NO_X versus time for control columns 20‰, planted, no wastewater (A) and 2‰, planted, no wastewater (B). Lines represent the mean at each depth.

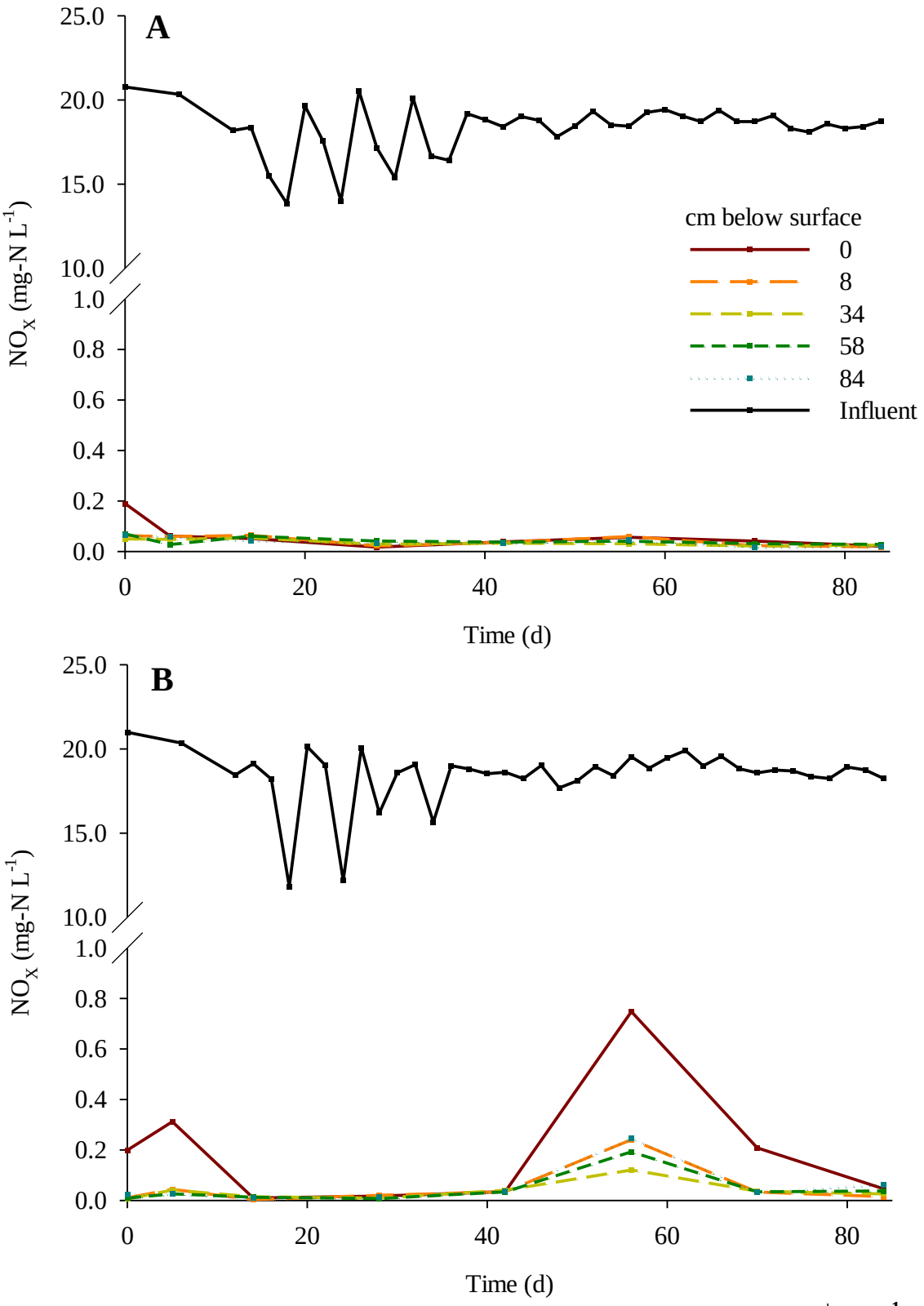

Figure B.7 Graph of NO_X versus time for columns 20‰, planted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (A) and 2‰, planted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (B). Lines represent the mean at each depth.

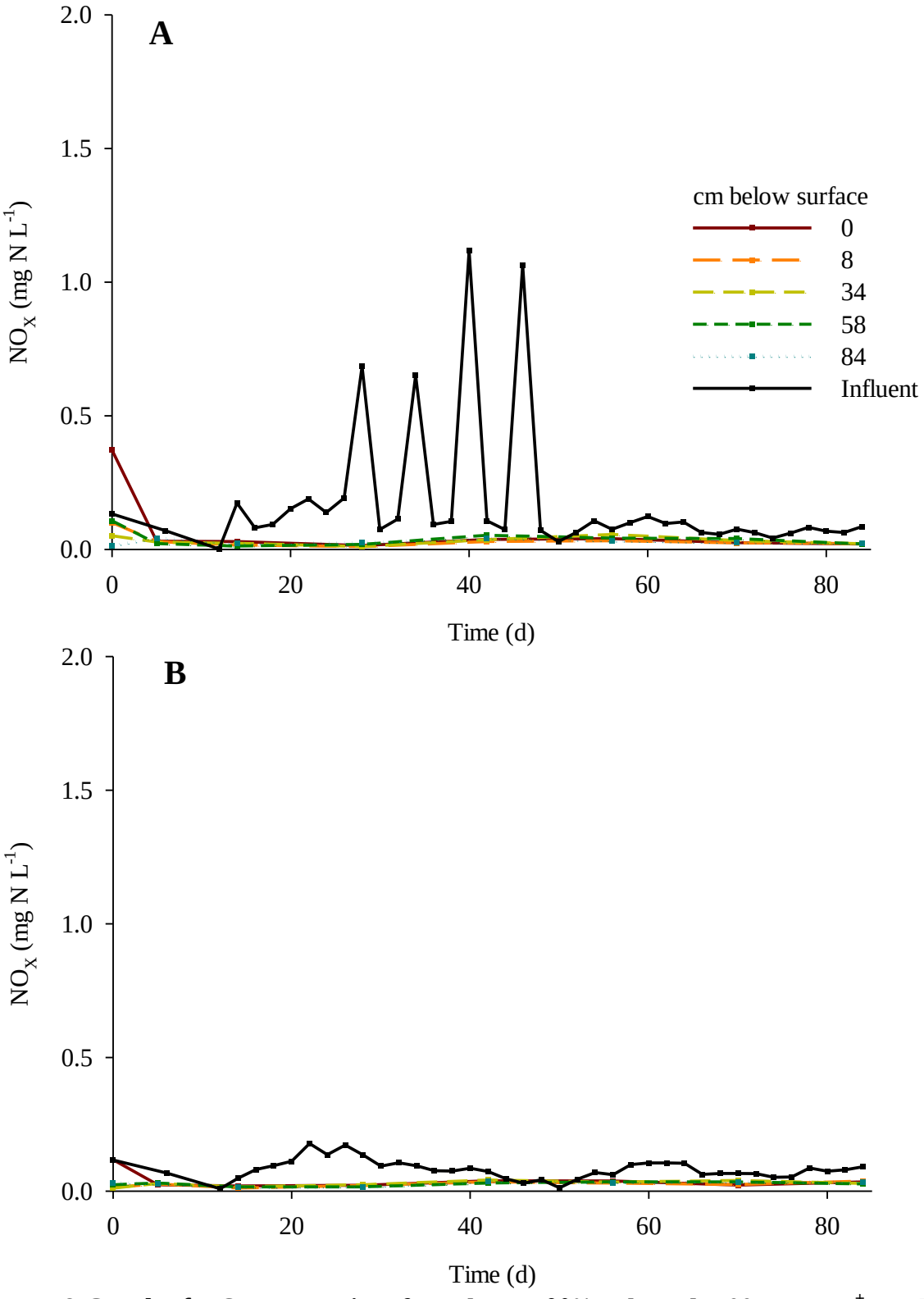

Figure B.8 Graph of NO_X versus time for columns 20‰, planted, 100 mg NH_4^+-N L^{-1} (A) and 2‰, planted, 100 mg NH_4^+-N L^{-1} (B). Lines represent the mean at each depth.

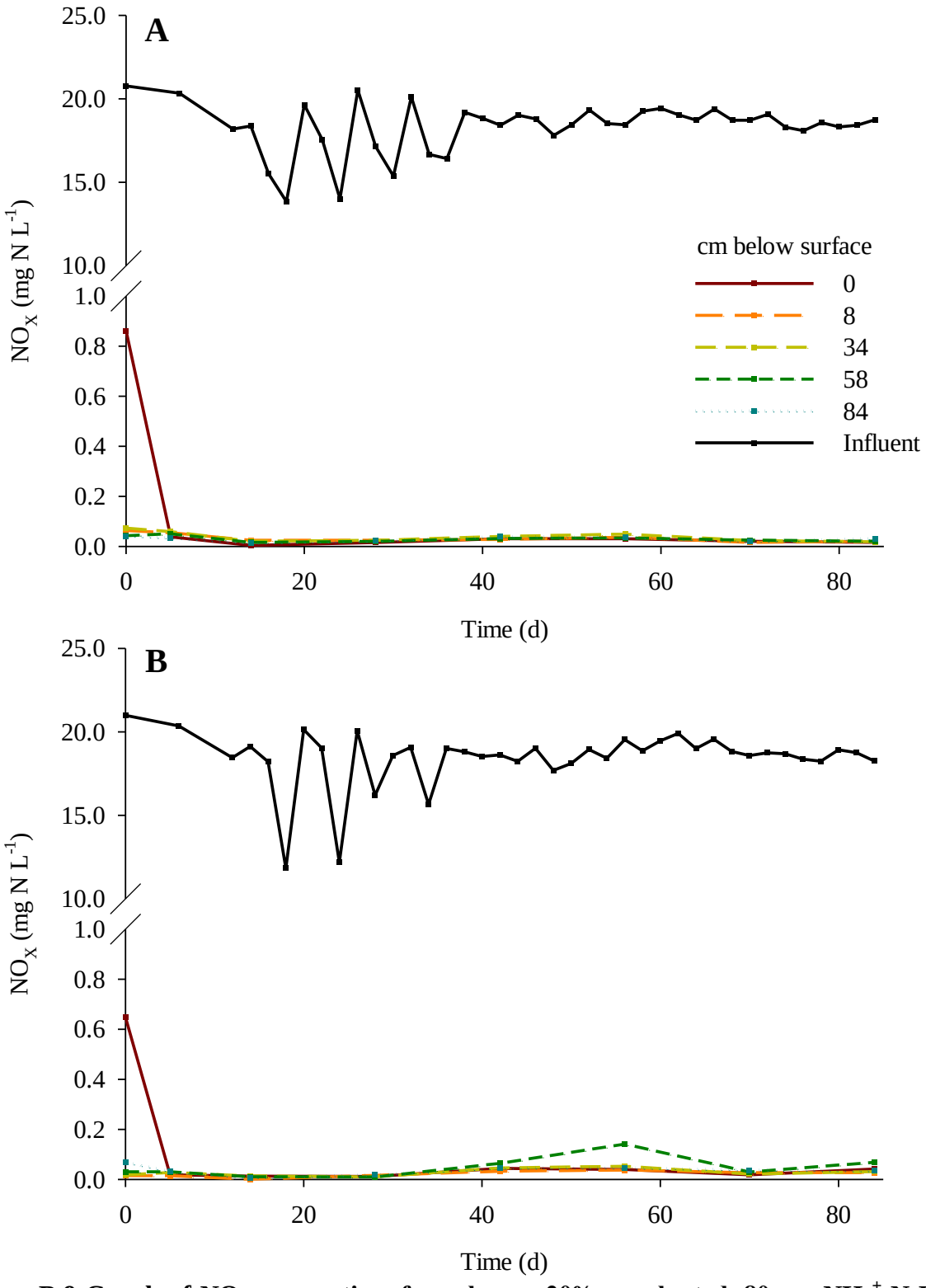

Figure B.9 Graph of NO_X versus time for columns 20‰, unplanted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (A) and 2‰, unplanted, 80 mg NH_4^+-N L^{-1}/20 mg NO_3^--N L^{-1} (B). Lines represent the mean at each depth.

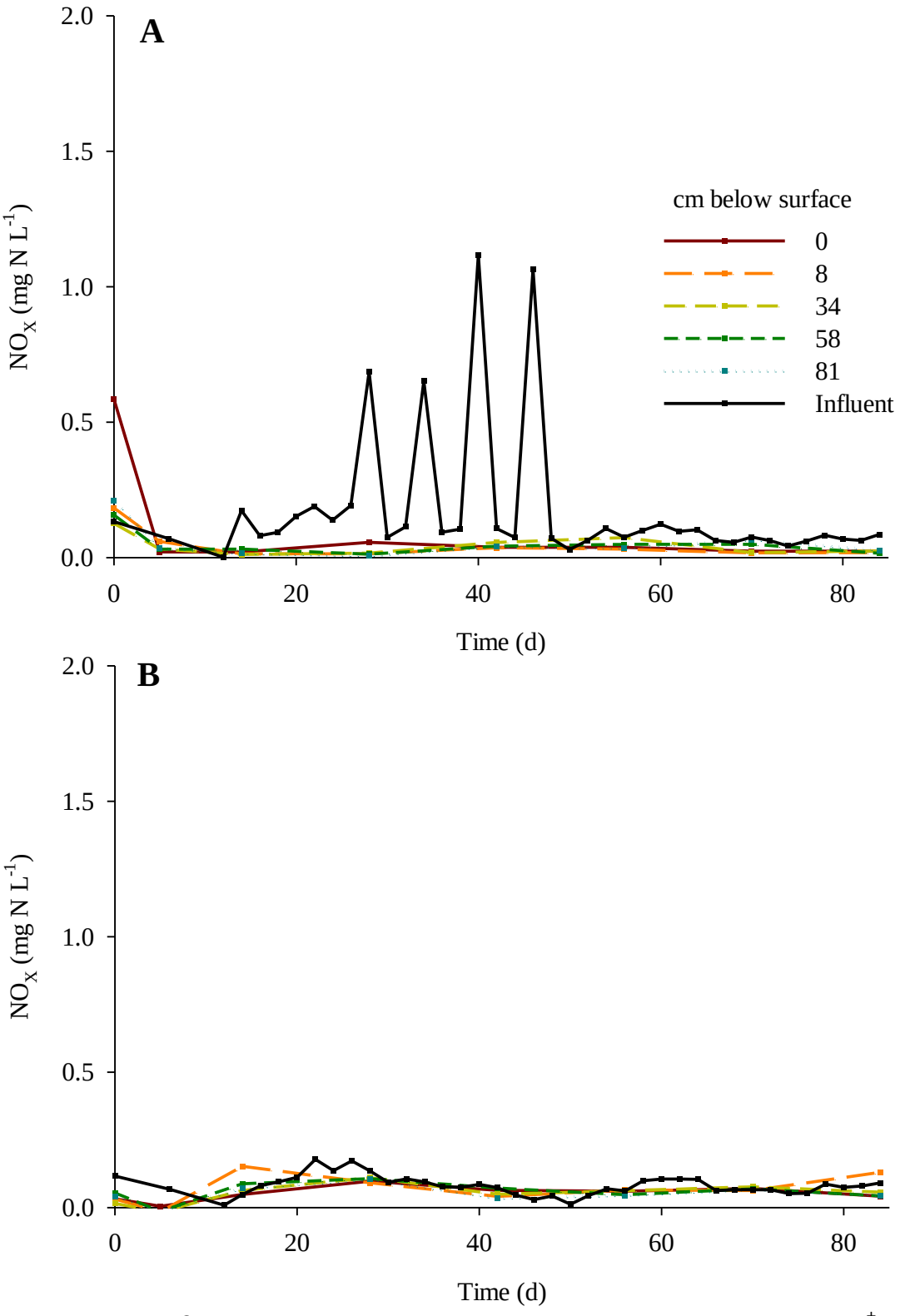

Figure B.10 Graph of NO_X versus time for columns 20‰, unplanted, 100 mg NH_4^+-N L^{-1} (A) and 2‰, unplanted, 100 mg NH_4^+-N L^{-1} (B). Lines represent the mean at each depth.

VITA

Lorna Anne Putnam was born in April 1980, in North Carolina. After many moves with her family, she ended up in Monticello, Kentucky, where she graduated from Wayne County High School in May 1998. She then moved to Lexington, Kentucky, and enjoyed some time as a Kentucky Wildcat at the University of Kentucky before graduating in August 2002 with a Bachelor of Art in chemistry and a Bachelor of Science in biology. Upon graduation she worked for two years as a research technician in two labs. Nine months were spent working in the lab of Dr. George Smith in the Physiology Department of the University of Kentucky School of Medicine. She then moved to Covington, Louisiana, where she worked in the lab of Dr. Bruce Bunnell in the Gene Therapy Division of the Tulane National Primate Research Center. During her time at Tulane, she decided to switch fields and began her studies in August 2004 as a fulltime graduate student in the Department of Oceanography and Coastal Sciences at Louisiana State University. She is currently a candidate for the degree of Doctor of Philosophy in the Department of Oceanography and Coastal Sciences, which will be awarded December 2009.