Seasonal nitrogen removal and the co-presence of exogenous carbon in constructed wetland mesocosms
by Kate Alexis Riley

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University

Abstract:
Constructed wetlands have emerged as an aesthetic, sustainable form of wastewater treatment. Though constructed wetlands have shown adequate levels of carbon removal in wastewaters, the remediation of secondary nutrients like nitrogen has been less successful. This research attempted to contribute performance and process-based information regarding seasonal nitrogen removal in the presence of carbon. Gravel-based column wetlands planted with Carex rostrata (beaked sedge), Typha latifolia (broadleaf cattail), and unplanted controls were monitored during plant dormancy and cold-temperature conditions (4°C), and during active plant growth and warm-temperature conditions (24°C).

A synthetic wastewater was applied to the columns in batch, for a series of 10-day incubations within each season. Ammoniacal-nitrogen influent levels were representative of municipal wastewater concentrations (40ppm). Exogenous carbon was applied to additional Carex columns in the range of 0-200ppm COD. Aqueous concentrations of carbon and nitrogen species were monitored throughout the incubations. Side experiments were also performed to clarify the role of internal nitrogen processes like sorption and plant and microbial assimilation. Select columns were traced with isotopic N15 and destructively sampled. A series of sorption tests were also performed on the gravel substratum.

Total nitrogen removals ranged from 62% to 94% in the planted columns, and 41% in the unplanted controls. The presence of plants positively influenced nitrogen removal during both seasons. Plant species effects were evident at 24°C, when Carex outperformed Typha. Overall nitrogen removal was not markedly affected by season or carbon load, though these factors implicitly caused a shift within the internal removal mechanisms.

Results were strongly influenced by the batch, drain-and-fill conditions of the columns. The mechanistic studies indicated that the stable performance was facilitated by surface phenomena like sorption and sequestration. This work corroborates other current research that emphasizes the anaerobic, fixed-film behavior of sub-surface flow wetlands. Permanent nitrogen removal is limited, and wetlands should be coupled with other treatment technologies for this purpose.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Constructed wetlands have emerged as an aesthetic, sustainable form of wastewater treatment. Though constructed wetlands have shown adequate levels of carbon removal in wastewaters, the remediation of secondary nutrients like nitrogen has been less successful. This research attempted to contribute performance and process-based information regarding seasonal nitrogen removal in the presence of carbon. Gravel-based column wetlands planted with *Carex rostrata* (beaked sedge), *Typha latifolia* (broadleaf cattail), and unplanted controls were monitored during plant dormancy and cold-temperature conditions (4°C), and during active plant growth and warm-temperature conditions (24°C).

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CHAPTER 1

INTRODUCTION

As primary consumers, perhaps it is fitting for humans to acknowledge the fate of our wasted products. Natural wetlands, in their latent richness, are the biogeographical culmination of degradation processes. Though humans have used wetlands as waste repositories for centuries, the purposeful manipulation of wetlands began in the 1950s with the advent of biological remediation. Constructed wetlands have been acclaimed as an aesthetic, low energy and maintenance alternative for mechanical wastewater treatments. But under what conditions are wetlands viable, and when are they thermodynamically impractical?

The second generation of constructed wetland research is actively pursuing these questions, as sanctioned design criteria are not available. This work attempted to observe nitrogen removal in constructed wetland mesocosms under two potentially limiting, but realistic conditions: carbon load, and the winter season, as characterized by cold-temperatures and plant dormancy. The feasibility of nutrient removal in cold climates has not been established due to a lack of operational data. The concurrent removal of carbon and nitrogen has been a historic challenge in wastewater treatment, and sub-surface flow wetlands are typically limited to 30-40% decrease in total nitrogen (Brix 1994). The United States Environmental Protection Agency (EPA) has identified nitrogen removal as a first-priority research topic, with attention to nitrogen transformations and removal mechanisms, and the effects of temperature and season on these processes (EPA 1993).

In addition to providing seasonal performance data, this research attempted to clarify the internal mechanisms and eventual fate of nitrogen constituents. Information regarding discrete processes and their interactions could resolve controversial issues, such as the true role of wetland plants, microbial transformations, and physical sedimentation. A phenomenological approach may also provide a basis for a generalized model, which could potentially incorporate the numerous variables that are specific to wastewaters, loading conditions, and regions – such as carbon load and seasonal cycles. Otherwise, constructed wetland design will continue to be limited to system-specific case studies and associated empirical correlations.

Though this work was performed on relatively young, pilot-scale mesocosms, the analysis incorporated several years of observations, and results from established, full-scale systems, to provide an integrated perspective of wetland behavior. The interpretation of results accounted for system effects, and aimed to correlate observations and relevance to applied constructed wetland systems.

Goals and Objectives

The constructed wetlands research project at Montana State University has been operating in a greenhouse for three growing seasons. The inclusive purpose of this project has been to supplement the available operational data of constructed wetlands in cold climates. For the first two years of the experiment, the system was loaded under conditions similar to a primary-treated municipal effluent with high influent concentrations of carbon, nitrogen, phosphate and sulfate. These parameters were tracked through a series of temperatures and plant growth conditions simulating temperate seasonal cycling. The results of this experiment suggested that the interdependence of these nutrients, especially nitrogen and carbon, significantly affected their removal efficacy (Biederman 1999). Nitrogen removal was hypothetically limited due to the lack of electron acceptors after preferential carbon oxidation, and the environmental instability for nitrifying bacteria.

The purpose of the present research was to 1) optimize the system for nitrogen removal, 2) determine the relative strength of potential nitrogen-removal pathways, and 3) elucidate performance differences due to plant species, temperature and season, and carbon load. This work attempted to develop both a performance-based and process-based approach, recognizing the need in present wetland research for more rational, integrated design criteria.

Background

Wetland Definition and Classification

Frequently bordering aquatic bodies, wetlands provide an ecological transition to unsaturated, terrestrial conditions. Wetlands are traditionally defined by their saturated soils and associated vegetation. Though the public has begun to recognize the intrinsic value of these ecosystems, over half of the natural wetlands have been altered or developed in the continental United States (Kadlec and Knight 1996). Questions have arisen whether these places should be preserved for their intrinsic value, or conservatively managed for human use within the balance of the system.

Due to conservation, impact, and operative issues, several types of constructed wetlands have been developed that combine the chemical and biological benefits of natural wetlands, with the hydraulics and application of engineered systems. These structures range from pilot-scale batch reactors, to bench-scale and full-scale continuous-flow systems. In sub-surface flow wetlands, the water level is kept at the surface of the substratum, in contrast to free surface-flow wetlands, which have an overlying water layer.

Present Regulatory and Research Status

Despite constructed wetlands' functionality, aesthetic benefits, and low energy and maintenance costs as compared with similar treatment systems, they have not been widely adopted as a treatment alternative. The EPA (1993) restricts wetland use to secondary or tertiary treatment, or for polishing and redundancy. Constructed wetland design and regulation has not been fully standardized, especially in regard to nitrogen removal. Only 50% of the wetlands in the North American Treatment Wetland Database had nitrogen listed as a permit criterion. The majority of those permits were based on ammonium, with effluent levels of 1-10mg/L NH_4^+-N; the remaining targeted total nitrogen levels at 2-7.5mg/L (Kadlec and Knight 1996).

Nitrogen compounds are of importance due to their role in the eutrophication of downstream waters and metabolic disruptions in aquatic biota and humans. Ammonium, NH_4^+, is a component of both urea and fertilizers, and may be found in municipal wastewater in concentrations up to 50ppm and in agricultural runoff up to several hundred parts per million. Nitrate, NO_3^-, is typically absent in raw municipal wastewater, but may be found in agricultural or point-source runoff, and may be produced within a treatment system as an intermediate degradation product. Significant concentrations of nitrogen have also been detected in landfill leachate and wastewaters from the petroleum industry (Kadlec and Knight 1996).

Design methods for nitrogen removal have previously been based on statistical correlations from operational wetlands (Gale *et al.* 1993; Kemp and George 1997). This type of integrated, stochastic modeling assumes that system data is representative of universal wetland behavior. Deterministic models based on theoretical mechanisms have also been developed (Kadlec and Hammer 1988; Martin and Reddy 1997; McBride and Tanner 2000), but calibration of these models with empirical data is poor. Both modeling techniques are limited by the lack of spatial and temporal data from steady-state, full-scale wetlands (EPA 2000). The majority of available data is from immature systems, which may result in mis-estimations of performance, especially in regard to nitrogen removal. Steady-state conditions may take from 2 to 10 years, depending on the extent of plant and microbial establishment (Nichols 1983). Until wetland behavior becomes better understood, and legitimate data become available, the design and regulation of systems will be limited. The EPA (2000) has suggested that these goals may be accomplished through a "plan for the design of studies", and the refinement of the North American Database that was established in 1994. The EPA has recognized both the stochastic and deterministic approaches in wetland research, but has endorsed neither one.

Nitrogen Removal Mechanisms

Numerous transformation and transport processes make the wetland nitrogen cycle dynamically complex (Fig.1). Aqueous ammonium can be sorbed to organic and inorganic substratum; immobilized into plant and microbial biomass; nitrified and denitrified; and/or volatilized as ammonia gas. Though sorption and immobilization may “remove” nitrogen from the aqueous phase, this nitrogen is held within the system’s short or long-term storage capacity, and may affect future performance. The only permanent removal pathways for ammonium are sequential nitrification/denitrification, volatilization of gaseous ammonia, and plant harvestation.

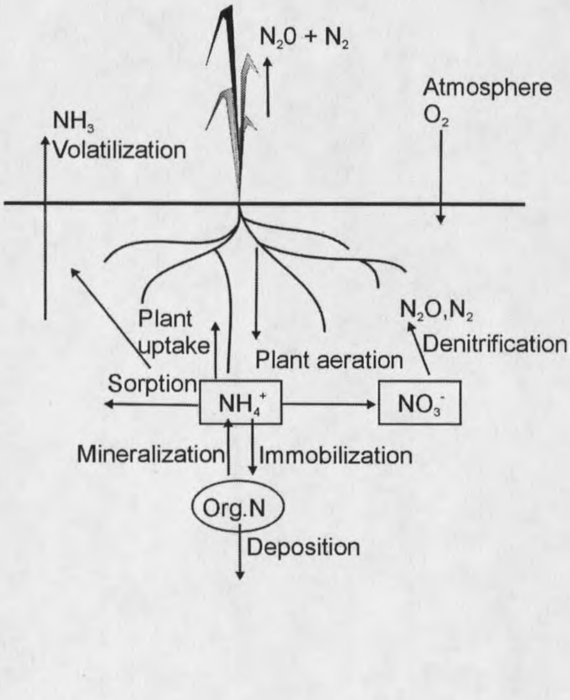

Figure 1. Nitrogen removal pathways.

The turnover time for the sequestered fractions to return to a soluble form differs with mechanism, season, and system characteristics. Sorbed, exchangeable ammonium represents the most labile form of stored nitrogen, as chemical equilibrium with the aqueous phase will be maintained. The growth and decay of microbial biomass may occur on the order of hours or days, and wetland macrophytes may take several seasons to decompose. A small fraction of recalcitrant plant and microbial matter may become unavailable, and permanently stored within the wetland. Though steady-state conditions are eventually expected in the vegetative and litter components, insufficient oxygen may cause nutrient accumulation and peat formation to occur indefinately, as has been documented in many natural wetlands (Kadlec 1989). Major processes in Figure 1 are addressed in more detail below.

Volatilization

Volatilization of un-ionized ammonia, $NH_{3(g)}$, may provide a nitrogen removal mechanism if $NH_{3(aq)}$ is present in significant concentrations. Aqueous NH_3 is in equilibrium with NH_4^+ according to the following reaction:

$$NH_{3(aq)} + H_20 = NH_4^+ + OH^- \qquad K_{eq}=10^{-4.6}$$

The amount of gaseous ammonia is in equilibrium with the aqueous concentration in accordance with Henry's law. However, for pH values less than 9.4, the amount of $NH_{3(aq)}$ in most wastewaters is negligible. As the measured pH in this experiment did not rise above 7.3, the expected percentage of total ammonia in the un-ionized form was 1.2

at 24°C (Kadlec and Knight 1996). Though ammonia stripping is initiated in other treatments by raising the pH, it is not a viable method in wetlands, and was not considered as a significant mechanism in this analysis.

Ammonium Sorption

Though sorption is usually deemed a finite, temporary sink for nitrogen in continuous-flow systems, this mechanism has gained attention in drained, or batch systems. Cationic ammonium may reversibly bind to surface sites on clay particles or iron and manganese oxides. The rapid kinetics of this reaction has been shown to affect the immediate removal of aqueous ammonium removal in gravel systems (Sikora *et al.* 1995). Equilibrium conditions between the surface and aqueous phases typically occur within a few days. The amount of sorbed ammonium at equilibrium is a function of the binding site and surface-water partition characteristics.

Ammonium sorption may be controlled through media and plant selection, and hydraulic format. Plant species that exhibit high root surface areas could enhance nitrogen removal in this manner (Tanner 1996). Specialized media such as zeolites have shown superior ammonium adsorption in wetlands (Green *et al.* 1996). Hydraulic conditions like batch-flow, or drain-and-fill operations regenerate surface sites. Since sorbed nutrients are not bio-available, cationic amendments such as Na+ may initiate desorption and subsequent bio-utilization (Green *et al.* 1996). However, the efficacy of these methods in amending overall nitrogen removal has not been adequately quantified.

Microbial Assimilation

Nitrogen is an essential element in all living cells. Immobilization of nitrogen into microbial biomass is driven by the catabolic need for carbon. Microorganisms may utilize ammonium or organic nitrogen, and their consumption represents a short-term storage mechanism for nitrogen in wastewater.

Immobilized nitrogen is predominantly in the form of amino acids, purines, and pyrimidines. Urea and uric acid are also produced as a metabolic by-product. These forms continuously undergo decay, hydrolysis, and the eventual transformation back into soluble, substrate NH_4^+-N. A portion (0-20%) of the assimilated nitrogen may remain in a recalcitrant form. In oxygen-depleted environments like wetlands, decay and product formation proceeds more slowly due to incomplete decomposition of carbohydrates, a low energy yield of fermentation, and a low nitrogen requirement of anaerobic microbes.

The substrata and hydraulic characteristics may also affect the fate of immobilized nitrogen. Cellular nitrogen may adsorb reversibly, or irreversibly to the proteinaceous conditioning film that surrounds gravel and roots (Characklis and Marshall 1990). Quiescent hydraulic conditions encourage the accumulation of nutrients at surfaces. Though constructed wetlands are not traditionally viewed as fixed-film reactors, cellular adhesion and accumulation may provide a legitimate source of nutrient removal in some systems. This technique has been used in other wastewater and water treatments, like trickling filters and packed bed reactors.

Wetland Macrophytes

Plant Assimilation. A substantial portion of influent nitrogen may also be assimilated by wetland macrophytes during their seasonal growth phase. Nutrient assimilation in plants is a metabolic process similar to that of the microbial cell. After an ion is absorbed, it is passed through cell membranes, and combined with carbon to form organic compounds. Oxygen is required in this process. Though the phosphorous concentration in this experiment and most wastewaters is within an acceptable range, this nutrient has also been found to limit nitrogen assimilation in other systems (Shaver and Melillo 1984).

Most plants prefer ammonium to other nitrogen species due to its energetic level. However, plants possess the enzymatic capability to reduce nitrate, which they will also assimilate. Equation 1 describes the vegetative uptake of ammonium and nitrate, using saturated-growth kinetics (Martin and Reddy 1997) (Eq.1):

$$J_{pl} = \frac{V}{Y} * u_{pl\max}\left(\left[\frac{(C_{NH4})}{(K_{NH4} + C_{NH4})}\right] * \left[\frac{(C_{NH4})}{(C_{NH4} + C_{NO3})}\right] + \left[\frac{(C_{NO3})}{(K_{NO3} + C_{NO3})}\right] * \left[\frac{(C_{NO3})}{(C_{NO3} + C_{NH4})}\right]\right)$$

where J_{pl}=plant uptake rate (g N/m$^{2}\cdot$yr)

V=plant mass per surface area (g plant/m^2)

Y=plant yield (g plant/g NH_4^+-N removed)

u_{plmax} = maximum plant uptake rate (1/yr)

$K_{NH4/NO3}$ = half saturation constants (mg/L)

C_{NH4}= ammonium concentration (mg/L)

C_{NO3} = nitrate concentration (mg/L)

Morris and Dacey (1984) estimated the NH_4-N and NO_3-N half-saturation constants for submersed *Spartina Altiflora* as 0.057 and 0.034 mg N/L, respectively. First order models have also been found to adequately represent laboratory data, with typical coefficients of 0.005 g N/ m^2 day (McBride and Tanner 2000).

Nutrient assimilation is affected by plant growth rate, below and above-ground biomass density, and tissue nutrient composition. Though tissue nutrient levels have been used to select certain plants for nutrient removal capacity, Tanner (1996) compared eight emergent macrophyte species and found that morphological and physiological differences were counterbalanced by growth rates. The average seasonal nitrogen uptake over all species was 30% of influent values on a mass basis. Several researchers have reported plant uptake to be the predominant removal mechanism, at 40-50% of applied total nitrogen (Breen 1990; Rogers *et al.* 1991; Koottatep and Polprasert 1997). However, these studies were performed on pilot-scale wetlands with relatively young plants, or low nutrient solutions. With increased nutrient loads, the efficiency of nitrogen uptake and biomass utilization declines. Wetlands may be considered eutrophic environments with excessive nutrient levels (EPA 2000). Consequently, under typical wastewater loads and full plant establishment, plant nutrient uptake is not a significant removal mechanism (Shaver and Melillo 1984).

<u>Fates of Plant Matter.</u> In temperate continental climates, wetland plants cease growth in the fall, and begin the natural progression of dieback, litterfall, leaching, and decomposition. Studies have shown that the decay of above-ground plant matter occurs in two phases; rapid decomposition of sugars and starches may occur within 30-60 days, followed by slower, exponential decay of celluloses (Bayley *et al.* 1985; Kadlec 1989).

Less information is available regarding roots and rhizomes, though a significant portion of below-ground matter also undergoes seasonal senescence and decay.

Harvesting plant matter may be used as an operational tool to enhance nitrogen removal and reduce the potential oxygen demand of the system (Garver *et al.* 1988; Koottatep and Polprasert 1997; Hosoi *et al.* 1998). Though harvesting plant material may remove 50% or more of the total biomass, Rogers *et al.* (1991) found only 33% of total assimilated nitrogen in harvestable aerial shoots due to translocation to roots. Plant harvesting also imparts a significant operational cost, which prohibits this practice of nitrogen removal in many wetlands.

Ancillary Plant Effects. Introducing plants into wetlands alters the physical, chemical and biological characteristics of the system. Plants mediate principal parameters such as alkalinity, pH, temperature, and water level. Plants improve the sorptive and filtration capacities of the substratum, provide aesthetic benefits, and encourage the presence of higher forms of biotic life. Wetland macrophytes establish diurnal and seasonal patterns, but also act as a buffer for short-term changes and cycles within the microbial community.

In addition to providing an explicit pathway for nitrogen removal through nutrient uptake, plants may indirectly promote nitrification through oxygenation of the root-zone (Gersberg *et al.* 1984; Reddy *et al.* 1989; Armstrong *et al.* 1992). However, other research has disputed the level of the plant aeration flux, and its role in nitrogen oxidation (Burgoon *et al.* 1995; Bodelier *et al.* 1997; EPA 2000).

Nitrification and Denitrification

Nitrification. Based on assumptions regarding available oxygen, many past studies have targeted the sequential nitrification/denitrification process as the primary mechanism for nitrogen removal in constructed wetlands (Gersberg *et al.* 1986; Reed and Brown 1995; Sikora *et al.* 1995). However, recent research questions the relative strength and reliability of this mechanism, which may exhibit high temporal and spatial heterogeneity (Breen 1990; Rogers *et al.* 1991; Farahbakhshazad and Morrison 1997).

Nitrification is the biological oxidation of nitrogen from its –3 oxidation state as ammonium, NH_4^+, to the +5 state as nitrate, NO_3^-. Nitrification includes the following primary reactions:

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_20 + NO_2^-$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

Two different consortia of microorganisms mediate these redox reactions to derive energy; ammonium-oxidizing bacteria include the genera *Nitrosospira, Notrosolobus, Nitrosomona, Notrosococcus, and Nitrosovibrio,* and the primary nitrite-oxidizing genera are *Nitrobacter* and *Nitrospira.* Most nitrifiers are gram-negative chemoautotrophic rods. Though specific species of heterotrophs and fungi have been found to nitrify, they are not believed to be prevalent in wastewater systems.

Nitrification is dependent upon pH, alkalinity, inorganic carbon source, redox, ammonium concentration, and temperature. In wetland environments, oxygen availability is the most crucial factor. Nitrification stoichiometrically requires 4.57 grams O_2 per gram of NH_4^+-N, and oxygen availability is limited due to the demand of plants, reduced chemicals, and heterotrophs. However, nitrifiers have been shown to persist in a variety of adverse conditions, including anoxic environments, and re-activate enzymatic processes when oxygen becomes available (Woldendorp and Laanbroek 1989).

Modeling Nitrification. Enzyme saturation kinetics may be used to theoretically describe the nitrification process. Ammonium oxidation is the limiting step and is used to model the complete reaction (Kadlec and Knight 1996).

$$J_{nitr} = \frac{V}{Y}\frac{(u_{nit\,\max} C_{NH4})}{(K_{nit} + C_{NH4})} \qquad \text{(Eq. 2)}$$

where J_{nitr}=nitrification rate (g NH_4^+-N/m^2·yr)
V=nitrifier mass per surface area (g /m^2)
Y=nitrifier yield coefficient (gm nitrifiers/g NH_4^+-N removed)
u_{nitmax} = maximum nitrifier growth rate (1/yr)
C_{NH4}= ammonium nitrogen concentration (mg/L)
K_{nit}= nitrification half saturation constant (mg/L)

Kadlec and Knight (1996) included the second limiting nutrient, dissolved oxygen, in a modified expression. The influence of temperature and pH have been empirically determined for attached growth systems (EPA 1993) and were also included:

$$J_{nit} = \frac{V}{Y} * 172e^{0.098(T-15)}[1 - 0.833(7.2 - pH)]\left[\frac{C_{NH4}}{C_{NH4}+1}\right] * \left[\frac{C_{DO}}{C_{DO}+1.3}\right] \qquad \text{(Eq. 3)}$$

where T= temperature (°C)
C_{DO}= dissolved oxygen concentration (mg/L)

Equations 2 and 3 may be simplified according to the ammonium concentration in relation to the half-saturation constant, K_{nit}. At 20 to 30°C, K_{nit} has been estimated between 1 to 10mg/L (Reddy and Patrick 1984). At low substrate concentrations, this reaction may be approximated as first-order; at high concentrations, a zero-order equation may be used. Operational data from wetlands is typically fit to first order kinetics, and an extensive review of regressed parameters may be found in Reddy and Patrick (1984).

Denitrification. Nitrate may accumulate in a system from nitrification or from influent concentrations. Current regulatory criteria for nitrate in drinking water supplies in the U.S. is 10mg/L. Due to its oxidation state, this ion is chemically stable and relatively diffuse. Nitrate may undergo several different fates, depending on redox conditions, biotic activity, and ammonium levels.

Denitrification is the biological reduction of NO_3^- to gaseous nitrogen products such as nitrogen gas (N_2), nitrous oxide (N_20), or nitric oxide (NO):

$$5(CH_2O) + 4NO_3 + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_20$$

In the absence of oxygen, denitrifiers oxidize carbohydrate substrates using nitrate as an electron acceptor. This reaction is performed by facultative, heterotrophic genera such as *Bacillus, Enterobacter, Micrococcus, Pseudomonas,* and *Spirillum.* In constructed wetlands, denitrifiers have been found in close association with nitrifiers in the root zone, with slightly higher populations (Ottova *et al.*1997).

In contrast to nitrification, denitrification raises the pH and produces alkalinity. This process is dependent upon temperature, oxygen availability, and the physical characteristics of the substratum. Systems that target nitrogen removal typically exhibit low exogenous carbon loads, which may also limit denitrification. Wetland macrophytes may supplement available carbon through root exudate and decomposition processes. Consequently, denitrification has been shown to follow the seasonal and diel trends of plant activity (Christensen and Ottengraf 1986). Though plant exudate is the most labile form of internally produced carbon, research has indicated that it is not produced in significant amounts, and has less effect than litter decomposition on denitrification rates (Brylinsky 1977; Sherr and Payne 1978).

Modeling Denitrification. Denitrification may be modeled using enzyme saturation kinetics with dual dependence on carbon and nitrate concentrations (Kadlec and Knight 1996):

$$J_{den} = \frac{V}{Y} * u_{den\,\max}\left[\frac{C_{NO3}}{K_{den}+C_{NO3}}\right]\left[\frac{C_{OC}}{K_{OC}+C_{OC}}\right] \qquad \text{(Eq. 4)}$$

where J_{den}=denitrification rate (g N/m^2·yr)
V=denitrifier mass per surface area (g /m^2)
Y=denitrifier yield coefficient (gm denitrifiers/g NO_3^--N removed)
u_{denmax} = maximum denitrifier growth rate (1/yr)
C_{NO3}= nitrate concentration (mg/L)
C_{oc} = organic carbon concentration (mg/L)
K_{den}= denitrification half saturation constant (mg/L)
K_{oc}= organic carbon half saturation constant (mg/L)

Experimental studies have estimated K_{den} between 0.1 to 0.2 mg/L, resulting in a zero-order nitrate dependence at concentrations above 1 to 2 mg/L. The half saturation constant of methanol has been estimated at 0.1mg/l, and does not usually limit denitrification kinetics (EPA 1993).

Denitrification from operational wetland data is most often described with first-order kinetics at both low and high nitrate concentrations (Gale *et al.* 1993). This result may be due to other processes that also affect nitrate disappearance in constructed wetlands. These mechanisms may include: chemodenitrification, assimilatory or dissimilatory reduction of NO_3^- to NH_4^+, and plant uptake.

Fate of Nitrogen Gases. Atmospheric nitrogen may be present in the bulk solution due to diffusion or rainfall, but the primary source of gaseous nitrogen in wetlands is from denitrification. The concentration of dinitrogen gas and nitrous oxide in the aqueous phase is proportional to the partial pressure in the atmosphere. However, dissolved nitrogen gas has a low reactivity, and research has indicated that a significant portion of nitrogen gases may be trapped in the soil, especially those not amended with macrophytes (Reddy *et al.* 1989). The products of denitrification are generally considered to be permanent and irreversible.

The Coupled Nitrification/Denitrification Process. The root surface provides the most likely location for coupled nitrification/denitrification (Fig.2). The efficiency of the coupled reaction is dependent upon the spatial pattern and concentration gradients between the oxidized and reduced regions, and the bulk water phase. Ammonium must first diffuse from the aqueous phase to aerobic sites, where it is converted to nitrate. If aqueous concentrations are low, ammonium may originate from the underlying sediment and undergo nitrification (McBride and Tanner 2000). In accordance with Fick's law, nitrate will either diffuse to anaerobic sites where denitrification could occur, or to the bulk solution phase where it will persist until the gradients change. Under oxygen-limited conditions, the availability of anaerobic sites may be high, but the chemical and charge demands of the bulk water phase may cause nitrate to diffuse out of the sediment (Jensen *et al.* 1993). Due to these possibilities, the coupled nitrification/denitrification process typically shows high variability in wastewater treatments (Grady *et al.* 1999)

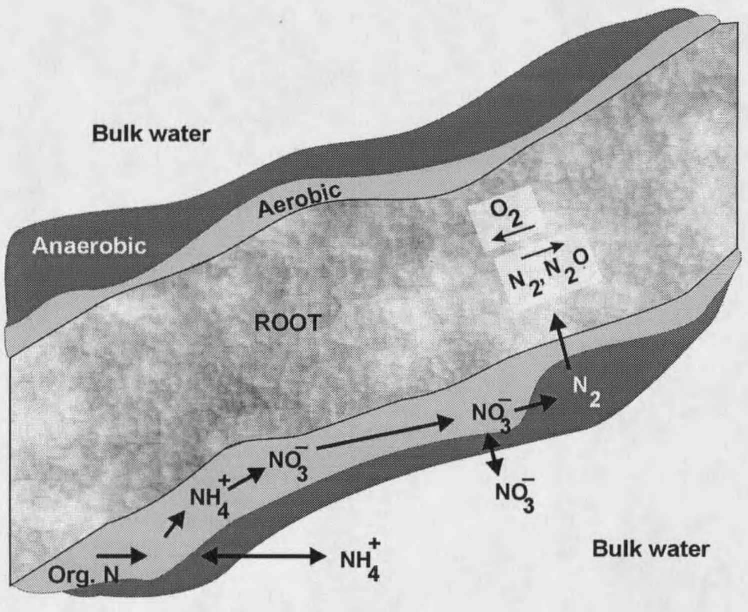

Figure 2. Coupled nitrification/denitrification process around a plant root.

Mechanism Interactions

Once nitrogen is introduced into a wetland, the kinetics, areal densities, and auxillary nutrient requirements of each mechanism will determine the mass balance. Plant uptake, microbial assimilation, and nitrification/denitrification are the primary pathways that were considered for the removal of ammonium in this experiment. The bioavailability of this element is limited foremost by physical transport and attachment to the gravel, root, or biofilm surfaces. In most wetlands for wastewater treatment, the concentration of nitrogen does not limit reactions. The controlling factor, and source of competition and interdependency between mechanisms lies in the relative affinity for other required parameters, such as carbon and oxygen.

Nitrogen Availability. Under sufficient oxygen and carbon availability, macrophytes, heterotrophs, and nitrifiers may directly compete for ammonium. However, the difference in time scale must be recognized; plants may capture an ion that has repeatedly cycled through microbial pathways, and then store it for several seasons. Plant assimilation is predominantly influenced by season, and microbes are strongly temperature-dependent. Both groups show high spatial variability.

While plants and nitrifiers may compete for ammonium, nitrifiers supply a more accessible and mobile source, NO_3^-, to macrophytes. Heterotrophs may also mediate nitrogen availability to plants through uptake of organic nitrogen and mineralization to ammonium (Kaye and Hart 1997). Under nitrogen limitation, microorganisms have been found to outcompete plants for ammonium and nitrate (Schimel *et al.* 1989). However, Bodelier *et al.* (1996) observed that plant nitrogen uptake negatively affected nitrifier activity, and imposed a more important stress on nitrifiers than heterotrophic competition for oxygen.

Carbon Availability. Organic carbon serves as the required electron donor for all heterotrophic organisms. Organic carbon promotes nitrogen removal through microbial assimilation and the denitrification process. Research has also suggested that carbon levels affect the relative nitrogen assimilation of plants and heterotrophs. Zhu and Sikora (1995) indicated that immobilization and denitrification were the largest removal mechanisms in a wetland loaded with exogenous carbon, in contrast to systems without carbon, in which plant uptake was the predominant mechanism. In addition to providing a labile, heterotrophic food source, carbon loads lower redox levels, and are inextricably linked to the oxygen demand of the system.

Oxygen Availability. Oxygen is arguably the governing parameter in the carbon and nitrogen cycle interactions. This element is a required electron acceptor for nitrifiers, plants, heterotrophs, and is the preferred source for denitrifiers and other facultative organisms. A sensitive balance must exist between nitrifying and denitrifying organisms, since both are needed for the coupled process to occur. In one wetland study, longer photoperiods and greater carbon availability stimulated the denitrifier, *Pseudomonas chlororaphis,* but significantly repressed the growth of nitrifying bacteria (Bodelier *et al.* 1997).

Similarly, performance data from batch studies suggests that nitrifiers are out-competed for oxygen by most heterotrophic organisms. Though oxygen half-saturation constants, K_m, of both heterotrophs and nitrifiers are similar, the consumption rate, V_{max}, of heterotrophs is much greater due to the higher biomass production per unit substrate consumed. The ratio V_{max}/K_m is typically used to determine the outcome for the competition for oxygen, and represents the specific affinity or substrate utilization rate of a community. This ratio is usually several orders of magnitude lower for nitrifiers than heterotrophs (Bodelier *et al.* 1996). In systems in which nitrification has been observed, the available oxygen either supports both populations, or the heterotrophic organisms are limited by carbon (Bodelier and Laanbroek 1997).

Modeling Wetland Zones

Though this work does not develop a nitrogen removal model per se, the presented theory and mechanistic studies may aid in the development of a deterministic model, as previously described. The complexity of these models requires assumptions regarding the spatial distribution of trophic zones. Many researchers have adopted a layered film approach around plant roots, consisting of an aerobic layer overlaid by an anaerobic film and the bulk water phase (Fig 2). Though chemical and microbiological stratification has been observed in other submerged systems such as lake sediments and flooded soils (Iversen and Blackburn 1981; Di Toro *et al.* 1990) and trickling filters (Revsbech *et al.* 1989), discrete gradients have not been observed in wetland biofilms (Conrad 1996). Microbial activity within biofilms is typically heterogeneous, and may be

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