



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
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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).

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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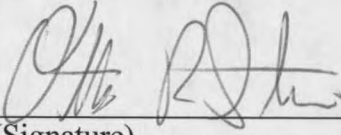
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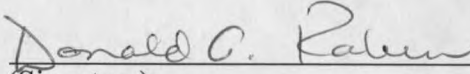
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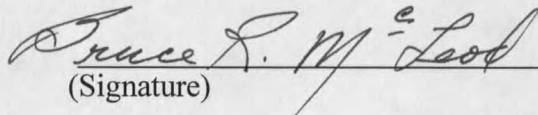
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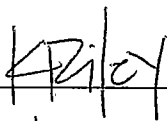

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ABSTRACT

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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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.

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 $\text{NH}_4^+\text{-N}$; 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.

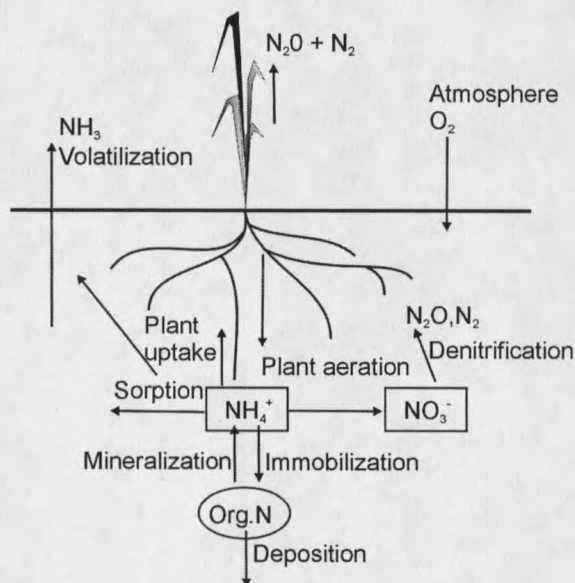
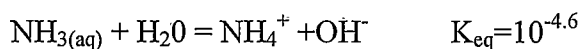


Figure 1. Nitrogen removal pathways.

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 indefinitely, 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, $\text{NH}_3(\text{g})$, may provide a nitrogen removal mechanism if $\text{NH}_3(\text{aq})$ is present in significant concentrations. Aqueous NH_3 is in equilibrium with NH_4^+ according to the following reaction:



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 $\text{NH}_3(\text{aq})$ 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 $\text{NH}_4^+\text{-N}$. 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/m²·yr)

V = plant mass per surface area (g plant/m²)

Y = plant yield (g plant/g NH₄⁺-N removed)

$u_{pl\ max}$ = 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 NH₄-N and NO₃-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 $0.005 \text{ g N/ m}^2 \text{ day}$ (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).

Fates of Plant Matter. 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.

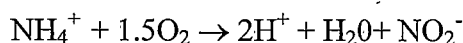
Ancillary Plant Effects. Introducing plants into wetlands alters the physical, chemical and biological characteristics of the system. Plants mediate principal parameters such as alkalinity, pH, temperature, and water level. Plants improve the sorptive and filtration capacities of the substratum, provide 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:



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).

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

where J_{nitr} =nitrification rate ($\text{g NH}_4^+\text{-N/m}^2\text{-yr}$)
 V =nitrifier mass per surface area (g/m^2)
 Y =nitrifier yield coefficient ($\text{gm nitrifiers/g NH}_4^+\text{-N removed}$)
 $u_{nit\ max}$ = maximum nitrifier growth rate ($1/\text{yr}$)
 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:

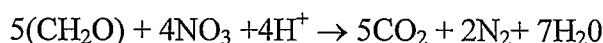
$$J_{nit} = \frac{V}{Y} * 172e^{0.098(T-15)} [1 - 0.833(7.2 - pH)] \left[\frac{C_{NH4}}{C_{NH4} + 1} \right] * \left[\frac{C_{DO}}{C_{DO} + 1.3} \right] \quad (\text{Eq. 3})$$

where T = temperature ($^{\circ}\text{C}$)
 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_2O), or nitric oxide (NO):



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):

$$J_{den} = \frac{V}{Y} * u_{den\ max} \left[\frac{C_{NO_3}}{K_{den} + C_{NO_3}} \right] \left[\frac{C_{OC}}{K_{OC} + C_{OC}} \right] \quad (\text{Eq. 4})$$

where J_{den} = denitrification rate (g N/m²·yr)
 V = denitrifier mass per surface area (g /m²)
 Y = denitrifier yield coefficient (gm denitrifiers/g NO₃⁻-N removed)
 $u_{den\ max}$ = maximum denitrifier growth rate (1/yr)
 C_{NO_3} = 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)

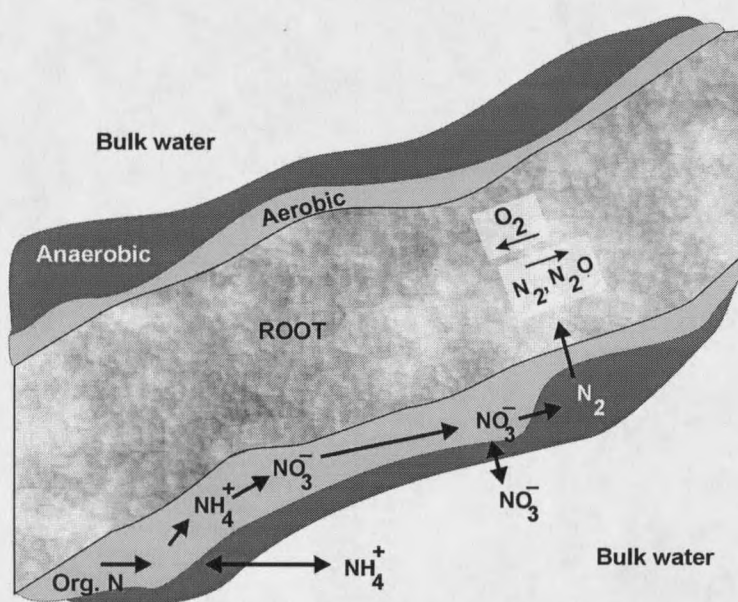


Figure 2. Coupled nitrification/denitrification process around a plant root.

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

difficult to mathematically simulate (Characklis and Marshall 1990; Stewart *et al.* 1995). In addition, in situ studies of nitrifying biofilms have identified interspersed microbial populations (Okabe *et al.* 1999), not necessarily limited by the anoxic and oxic regions of the film (Schramm *et al.* 1996). Consequently, the layered biofilm models may not be completely representative of wetland behavior.

However, distinct redox zones have been observed within the volume of the wetland bed due to the overall effects of atmospheric and rhizosphere oxygenation, deposition of litter, and hydraulic flow. Models have been created that spatially delineate these aerobic and anaerobic regions, and their associated nutrient transformations (Kadlec and Hammer 1988; McBride and Tanner 2000). As in a biofilm model, diffusion is the rate-controlling parameter. Martin and Reddy (1997) observed that considerable increases in model values for mineralization, nitrification, denitrification, and vegetative uptake rates did not significantly affect overall nitrogen removal. Conversely, manipulations in the diffusion rates of oxygen and nitrate markedly changed performance. To attain agreement with experimental data, literature diffusion coefficients were greatly increased, which Martin and Reddy (1997) rationalized as a consequence of plant transpiration. This result would suggest that further information regarding oxygen transport rates and mechanisms may particularly foster process-based nutrient removal models.

Alternative Wetland Designs

Due to the importance of oxygen diffusion, alternative hydraulic designs have been considered for potential aeration capacity and nutrient removal. As in this

experiment, a drain-and-fill regime may oxidize substratum surfaces during interim periods between loading. Examples of other experimental treatments include: intermittent flooding and water level fluctuation (Tanner *et al.* 1999), and vertical up or downflow recirculation (Laber *et al.* 1997). Aerators or bubble air diffusers have also been used in constructed wetlands (Green *et al.* 1997), and two-stage reactors may reduce the carbonaceous oxygen demand in the first cell for potential nitrogen removal in the second cell (Kemp and George 1997). However, these manipulations require significant operational cost, and depart from the original goal of self-sustaining wetlands.

Literature Summary

Today, constructed wetlands are highly attractive to the public sector. Even in technical literature, wetlands are still somewhat viewed as a novel panacea. With the increased demand for full-scale implementations, scientists and regulatory agencies must acknowledge system limits and intrinsic variability. Misconceptions regarding potential performance and mechanisms have already resulted in failed designs (EPA 2000). Process-based research could potentially elucidate controversial issues, like rhizosphere oxygenation and nutrient transformation, but must also provide the practical, design-based criteria that are currently needed.

CHAPTER 2

MATERIALS AND METHODS

Experimental Design

The constructed wetland mesocosms used for this study were created in April 1997 and housed in Montana State University's Plant Growth Center (Allen 1999). Mature *Typha latifolia* (broadleaf cattail) and *Carex rostrata* (beaked sedge) were collected in their dormant state from natural wetland habitats near Bozeman, MT. The rhizomes were stored in cold (1°C), dark conditions for one month, washed, and placed in columns filled with gravel. Unplanted control columns were also established with pea gravel. The columns were initially filled with a standard nutrient solution (Peter's 50ppm N 20-10-20, Scotts Co.) and were maintained for 5 months with periodic draining and refilling with fresh nutrient solution.

The columns were constructed from 60cm x 20cm polyvinyl chloride pipe (PVC) and filled to a 50cm depth with washed pea gravel (0.5-1.5cm). The initial porosity was 0.27 and pore volume, 4.3L. Solution sampling tubes (0.3 cm diameter vinyl tubing) were installed vertically at depths of 5, 15, and 30 cm. Platinum redox probes were cased in PVC access tubes (1.1 cm diameter) at the same depths. To compensate for evaporative losses, tap water was continuously delivered to a pipe hydraulically connected to the column, which maintained the water level at the gravel surface (Figure 3).

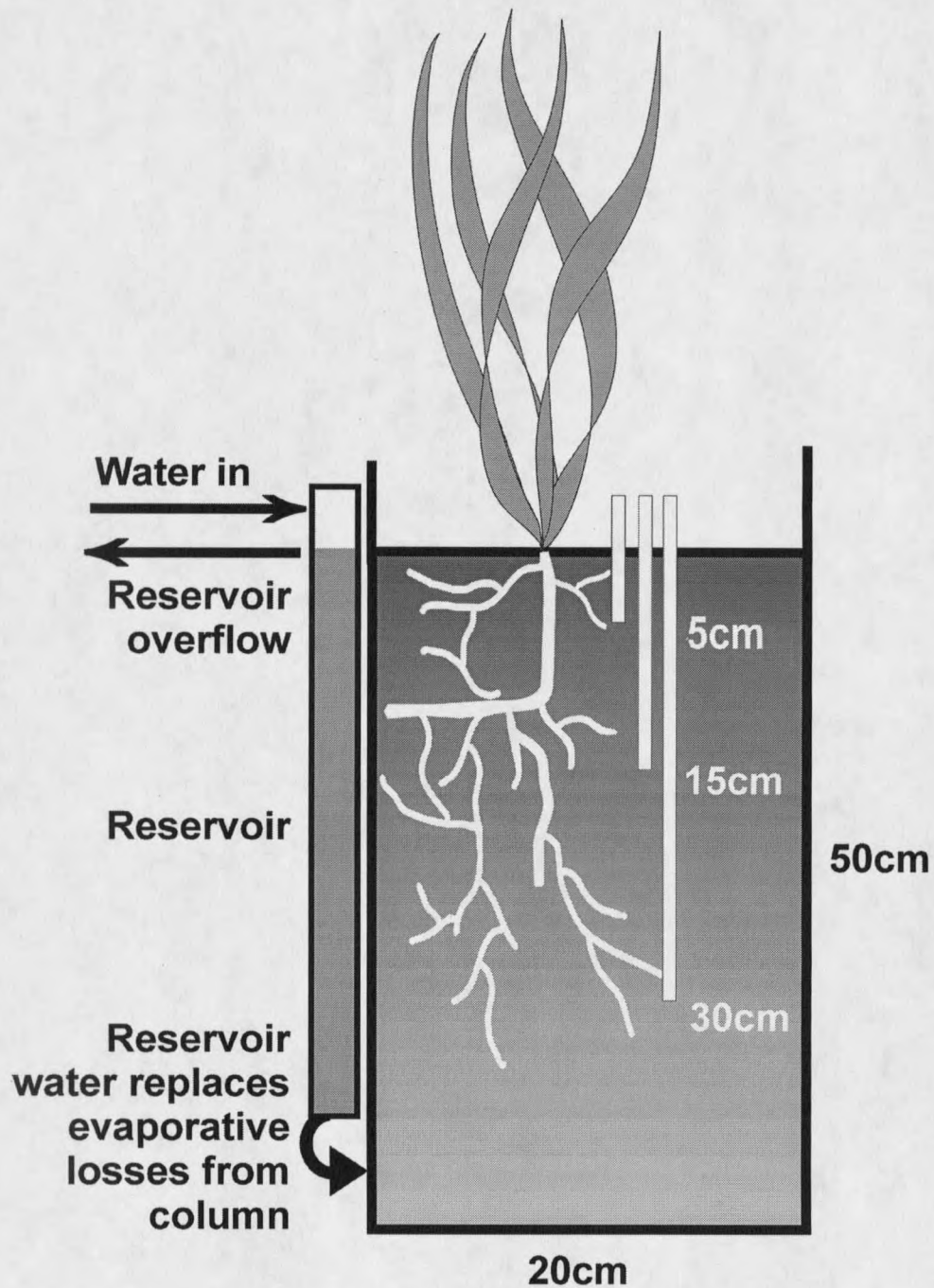


Figure 3. Schematic of column and water delivery system (Allen 1999).

The seasonal conditions of Montana were approximated within the constraints of the greenhouse. Natural light was used, and the relative humidity ranged from 30 to 70%. The cumulative daily net solar radiation ranged from 1 to 8 MJ m⁻² d⁻¹ (Towler 1999). From May 1997 to May 1999, the ambient air temperatures were stepped at 4°C intervals from the coldest condition in January and February at 4°C to the warmest temperature, 24°C, during July and August (Fig. 4). Initial research used these mesocosms to analyze the temperature and seasonal effects of the wetland receiving a wastewater high in carbon, sulfate, and nitrogen. Portions of these results (Allen 1999; Biederman 1999) were included in this analysis.

During the current experiment of May 1999 to September 2000, wetland nitrogen removal performance was observed for two different wastewater formulations; one without exogenous carbon, and one with a low-carbon load as compared to the previous study. Data was principally collected at the minimum temperature of 4°C, during plant dormancy, and at the maximum temperature of 24°C, during active plant growth (Fig.4). These temperatures were spanned with primary steps of 10°C, with short intermediate steps of 5°C between each interval. The primary temperature levels ran 2 months in duration, in which two or more 10-day wastewater incubations were completed. Side experiments were performed for mechanistic information, including the application of two inhibitors and an isotope tracer, and a series of sorption studies.

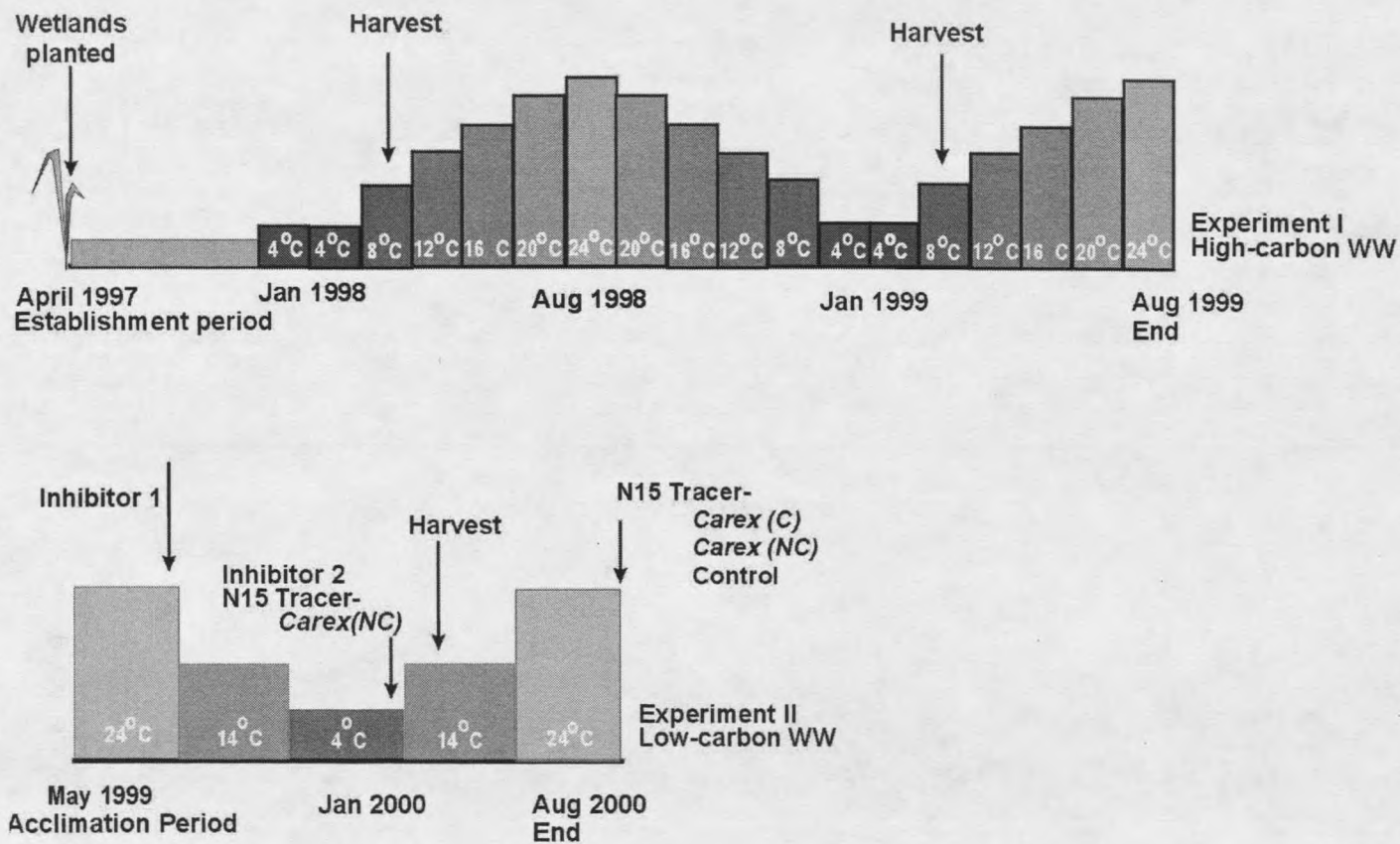


Figure 4. Timeline for the constructed wetland columns. Data prior to August 1999 from Allen (1999).

Treatments consisted of four, randomly arranged columns each of *Carex rostrata*, *Typha latifolia*, and unplanted controls receiving a wastewater without exogenous carbon. The wastewater of four additional *Carex rostrata* columns was amended with 200 mg/l $C_{12}H_{22}O_{11}$ as a carbon source. These synthetic wastewater compositions were intended to represent a wastewater that had undergone primary and secondary treatment to remove either all, or a portion of the influent organic carbon. The constituents included: 40mg/L NH_4-N as NH_4Cl , 7.89 mg/L PO_4-P as K_2HPO_4 , 0.21mg/L $FeCl_3$, 8.42mg/L H_3BO_3 , 1.89mg/L KI, 1.89mg/L $CaCl_2$, 4.0mg/L Na_2MoO_4 , 0.53mg/L $CuCl_2$, 9.16mg/L $MnCl_2$, and 6.63 mg/L $ZnCl_2$. The measured and theoretical influent parameters for the high-carbon, low-carbon, and no-carbon wastewaters are listed in Table 1. Within this discussion, the *Carex* carbon and no-carbon treatments are suffixed by (C) and (NC), respectively.

Parameter (mg/L)	High C		Low C		No C	
	Meas.	Theor.	Meas.	Theor.	Meas.	Theor.
COD	492 ± 4		117 ± 17	225	8 ± 2	0
TOC	140 ± 0	176	38 ± 12	84	4 ± 2	0
TN	43 ± 1	44	40 ± 1	40	38 ± 1	40
NH4-N	17.8 ± 0.3	17.2	39.5 ± 1.5	40.0	39.0 ± 0.9	40.0
ON	25	27	0	0	0	0
NO2/3	0	0	0	0	0	0
P04-P	8.3 ± 0.2	8	4.9 ± 0.3	8	4.8 ± 0.1	8
S04-S	14.1 ± 0.3	12	2.9 ± 0.3	0	2.7 ± 0.3	0
C/N	3.3	4.0	1.0	2.1	0.1	0.0
N/P	5.2	5.5	8.6	5.0	8.3	5.0
PH	6.9		7.0 ± 0.1		6.7 ± 0.3	

Table 1. Measured means and theoretical values for influent wastewater ± one standard error. Values taken over all columns and incubations. Data from previous experiment taken from Biederman (1999).

Sampling Method and Chemical Analysis

The columns were drained from the base. Upon emptying, fresh wastewater was immediately added from the top. The wastewater was mixed one day prior to the incubation to allow equilibration with the air temperature in the greenhouse. No vertical gradients were evident in preliminary sampling data, so samples were taken at the 15cm depth only. A 60mL syringe was used to extract the sample, which was immediately analyzed or preserved for further analysis. Sampling occurred at times 0, 0.3, 0.6, 1, 3, 7, and 10 days. A bromide tracer test indicated that a dilution factor of 5% would account for the porewater dilution of influent concentrations (Allen 1999).

The initial experimental design included two 10-day incubations within each of the 4 and 24°C temperature cycles. An additional incubation was performed as part of the inhibition study during the 4°C cycle. The experimental design was revised during the 24°C cycle due to the reduction of column replicates; two unamended *Carex* columns died, and one *Carex* was destructively sampled during the winter (Table 2). The five remaining *Carex* columns were used in sequential batch incubations of the no-carbon, and carbon amended wastewater. The seasonal influences were mitigated within the 24°C growing season by running one no-carbon incubation, followed by two carbon cycles, then the last no-carbon incubation. A pre-application of the specific wastewater occurred three days prior to each cycle to promote microbial establishment. Data from the unplanted controls and *Typha* columns were collected throughout the 4 incubations at 24°C.

Table 2. Experimental design for previous (Allen 1999) and current experiment. Values indicate column numbers. Asterisks designate columns traced with N¹⁵ and destructively sampled. Carbon amendment denoted by suffix (C) and unamended treatment by (NC).

1997-1999											
4°C						24°C					
<u>Incubation 1</u>			<u>Incubation 1</u>			<u>Incubation 1</u>			<u>Incubation 1</u>		
<u>Carex</u>	<u>Typha</u>	<u>Control</u>	<u>Carex</u>	<u>Typha</u>	<u>Control</u>	<u>Carex</u>	<u>Typha</u>	<u>Control</u>	<u>Carex</u>	<u>Typha</u>	<u>Control</u>
4	8	9	4	8	9	4	8	9	4	8	9
10	11	14	10	11	14	10	11	14	10	11	14
22	12	28	22	12	28	22	12	28	22	12	28
23	26	31	23	26	31	23	26	31	23	26	31
<u>Incubation 2</u>			<u>Incubation 2</u>			<u>Incubation 2</u>			<u>Incubation 2</u>		
4	8	9	4	8	9	4	8	9	4	8	9
10	11	14	10	11	14	10	11	14	10	11	14
22	12	28	22	12	28	22	12	28	22	12	28
23	26	31	23	26	31	23	26	31	23	26	31

1999-2000											
4°C						24°C					
<u>Incubation 1</u>				<u>Incubation 1</u>				<u>Incubation 2</u>			
<u>Carex</u>	<u>Carex</u>	<u>Typha</u>	<u>Control</u>	<u>Carex</u>	<u>Carex</u>	<u>Typha</u>	<u>Control</u>	<u>Carex</u>	<u>Carex</u>	<u>Typha</u>	<u>Control</u>
(C)	(NC)			(C)	(NC)			(C)	(NC)		
4	3	2	6	-	4	2	6	4	-	2	6
10	15	21	7	-	10	21	7	10	-	21	7
22	17	25	18	-	15	25	18	15	-	25	18
23	20	30	24	-	22	30	24	22	-	30	24
					23			23			
<u>Incubation 2</u>				<u>Incubation 3</u>				<u>Incubation 4</u>			
4	3	2	6	4	-	2	6	22*	4	2	6
10	15	21	7	10	-	21	7		10	21	7
22	17	25	18	15	-	25	18		15	25	18
23	20*	30	24	22	-	30	24		23*	30	24*
				23							

Aqueous samples from all columns were immediately analyzed for carbonaceous oxygen demand (COD; 0-1500mg/L) and total nitrogen using colorimetric procedures (Hach Co., Loveland, CO). Total organic carbon samples were acidified with 20% H_3PO_4 and later measured using a Dohrmann (model DC-80) carbon analyzer. All other procedures were performed on preserved samples that were filtered with a 0.20 μm cellulose acetate filter and stored in sterile test tubes at 2°C. Ammonium was determined using the modified Berthelot colorimetric method; nitrate, sulfate, and phosphate were measured using high performance liquid chromatography (Dionex Co.). Ammonium, nitrate, phosphate, sulfate, and COD were measured for all sampling times; pH, total organic carbon and total organic nitrogen were measured throughout the first incubation of each temperature cycle except at 0.3, and 0.6 days, and only at days 0 and 10 of the other incubations.

Inhibition Analysis

Two different inhibitors were used in an attempt to quantify nitrification potential. Unfortunately, both applications were unsuccessful. Chlorate, a compound that is specifically toxic to nitrite-oxidizing bacteria, was applied to half of the columns in each treatment at 10mM concentration during the preliminary study period in August 1999 as described by Wagner *et al.* (1995). Nitrite (NO_2^-), an intermediate product of the nitrification process, was then measured colorimetrically on all columns (Hach Co., Loveland CO). However, no apparent difference was found between the inhibited and non-inhibited treatments. Nitrate was periodically detected with ion chromatography, so

nitrification was occurring, and the inhibitor was ineffective. Therefore, N-serve (2-chloro,6-tichloromethyl pyridine), an inhibitor which targets ammonia oxidizing enzymes, (Campbell and Aleem 1965) was applied in January 2000. The ammoniacal nitrogen concentrations of the inhibited treatment were compared to the non-inhibited case, with no measurable differences.

Though few biological inhibitors are completely effective, the inadequacy of the two applications was most likely due to sorption or sequestration of the compounds, diffusion limitation into microbial biofilms, or the mis-estimation of the necessary threshold concentrations that are usually documented for soil or aquatic systems.

Isotopic Analysis

Further investigation of the nitrogen cycle was completed with additional sampling and the use of isotopic nitrogen. N^{15} was added as ammonium chloride comprising 50% of the total ammonia nitrogen on a molar basis. The purity of the isotope was 99.9% (CDE Isotopes). One auxiliary *Carex* column was labeled and destructively sampled at the end of the preliminary study in August 1999. An operational *Carex* *rostrata* column was labeled and destroyed at the end of the last 4°C incubation in January 2000. During the final 24°C cycle in August 2000, one column each of *Carex* with and without carbon amendment, and one unplanted control were traced and destructively sampled.

Aqueous samples from the N^{15} labeled columns were taken at the beginning and end of the incubation and analyzed for isotopic nitrogen as NH_4^+ -N and NO_3^- -N. On day 10, the columns were taken to the laboratory, drained, and the PVC pipe was sawed longitudinally to recover the column profiles. The above and below-ground plant matter were manually separated, dried at 90°F, and weighed. The gravel was dried at room temperature and weighed.

Sorbed ammonium was extracted from submersed surfaces with 2N KCl (Bremner and Mulvaney 1981). Triplicate 7-8 gram samples of below-ground plant material and 50 gram gravel samples were shaken overnight and centrifuged at 6000 rpm for 20 minutes. The supernatant was then filtered through 1 μ m filters and stored at 2°C. Assimilated organic fractions of N^{15} were determined in triplicate for the above and below-ground plant matter. After homogenization, 0.2g sub-samples were digested in 4mL sulfuric acid using the Hach digestion method for total Kjeldahl nitrogen. Several methods were employed to determine the organic N^{15} fractions within the biofilms surrounding the gravel, with discouraging results. The organic nitrogen biofilm fraction on roots and rhizomes were included in the plant digestion, but remained unmeasured for the gravel.

The aqueous samples, KCl extracts, and plant tissue digests were then analyzed for ammonia nitrogen using the mason-jar diffusion method (Mulvaney 1997a).

Ammonium in the sample diffused into a boric acid indicator solution within a suspended petri dish contained in the sealed jar. Ammonium levels in the boric acid solutions were determined by titration with 0.005M sulfuric acid. Samples were prepared for isotopic

analysis by removing the boric acid with heat, methanol, and distilled water, until ammonium sulfate crystals remained. The crystals were dissolved in distilled water, transferred to a microtiter plate, and dried at 90°F. The samples were shipped to the N¹⁵ Analysis Service at University of Illinois – Urbana for analysis via the Rittenberg technique on an automated mass spectrometer. The analytical precision of the procedure was 0.001 atom % N¹⁵ (Mulvaney 1997b).

Sorption Analysis

Three separate sorption experiments were also performed (Breen 1990, Sikora *et al.* 1995), in addition to the analysis of N¹⁵ sorbed on the substratum. The purpose of the first trial was to determine the time to steady state for the initial sorption of ammonium. Four replicates each of 40 mg/L and 5 mg/L NH₄-N (100mL) were shaken with 100g gravel taken from a destructively sampled column. The aqueous solutions were sampled through time, filtered through a 0.2 micron filter, and analyzed colorimetrically. Secondly, the maximum sorptive capacity of the gravel was determined using duplicate NH₄⁺-N concentrations ranging from 0 to 6000 mg/L. The bottles incubated in the dark for the equilibration time, and the sorbed NH₄⁺-N was extracted using 1N KCl. The bottles were shaken overnight, and the supernatant was filtered and tested for NH₄⁺-N. The third sorption test used concentrations in the range of the influent wastewater, 0-40ppm NH₄⁺-N, using the same extraction and sampling procedures as described.

Data Analysis

Linear models were developed using the software program Minitab (1995) through analysis of covariance, which combines the statistical features of regression and analysis of variance. A general format is represented by:

$$Y = \mu + \text{Tmt} + \text{Temp} + \text{Tmt} * \text{Temp} + \beta * \text{Day} + \varepsilon \quad (\text{Eq. 5})$$

Where: Y= predicted response

μ = mean

Tmt, Temp= possible wastewater or plant treatment, temperature predictor

Tmt*Temp= possible interaction between treatment and temperature

β = regression coefficient

Day= possible covariate, day of batch incubation

ε = residual

Treatment effects were statistically evaluated using Tukey's multiple comparison procedure for all temperature and treatment combinations, at a confidence level of 95%. This technique's level of significance is based on a family of inferences simultaneously evaluated, and may be more conservative than individual comparisons (Neter *et al.* 1996). This method was used to estimate the effects of wastewater treatment, plant, and season, on the aqueous phase concentrations of ammonium and COD. Sulfate levels were monitored to assess oxidation status. A carbon-nitrogen interaction model was also developed for the *Carex* treatment to evaluate the effects of influent COD levels on the amount of aqueous ammonium removal. These linear models used observations from day 1 through 10 for all incubations of a given season. Observations less than day 1 were analyzed separately using analysis of variance.

The complex profiles of nitrate with time required further manipulation. A $\log_{10}$ transformation was performed on the response, NO_3N . The detection limit of the analytical test was substituted for zero values in this calculation. The response NO_3N and was analyzed separately for days 0.0, 0.3, 1, 3, 7, and 10 using analysis of covariance. The geometric mean, or the antilog of the model-predicted mean was used in the presentation of the results. The standard error was approximated using the delta method (Rice 1988).

Constituents that were not present in the influent, such as nitrate and sulfate, were analyzed on a mass concentration basis. The removal ratio, C/Co , was used to normalize the influent variability of ammonium and COD. Temperature and seasonal effects were not statistically separable, and were considered a single environmental variable in the analyses.

CHAPTER 3

ResultsTotal Nitrogen Removal

Total nitrogen was predominantly in the form of $\text{NH}_4^+\text{-N}$ throughout the incubation time (Fig.V). The seasonal, wastewater treatment, and plant species effects on total nitrogen removal were similar to ammonium trends, which are further presented. The dissolved and particulate organic nitrogen in the aqueous phase, as calculated by the difference between total nitrogen and inorganic values ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) typically ranged from 2-5mg/L.

Ammonium Removal

The removal of aqueous ammonium is shown in Figure V. Relative treatment performances were established within 24 hours with the immediate, curvilinear depletion of ammonium (Table III). After day 1, ammonium concentrations did not markedly change with time, and incubation day was not a significant predictor in the model (Eq. 5). Overall performance trends were established using observations after day 1.

Seasonal effects were displayed only in the *Carex (NC)* treatment ($p=0.00$), with higher removal of $\text{NH}_4^+\text{-N}$ during summer conditions at 24°C ($C/C_0 = 0.05 \pm 0.02$) than during the winter at 4°C ($C/C_0 = 0.25 \pm 0.03$). In contrast, the carbon-amended *Carex (C)* columns tended to decline in performance from a removal ratio of 0.02 ± 0.03 in winter

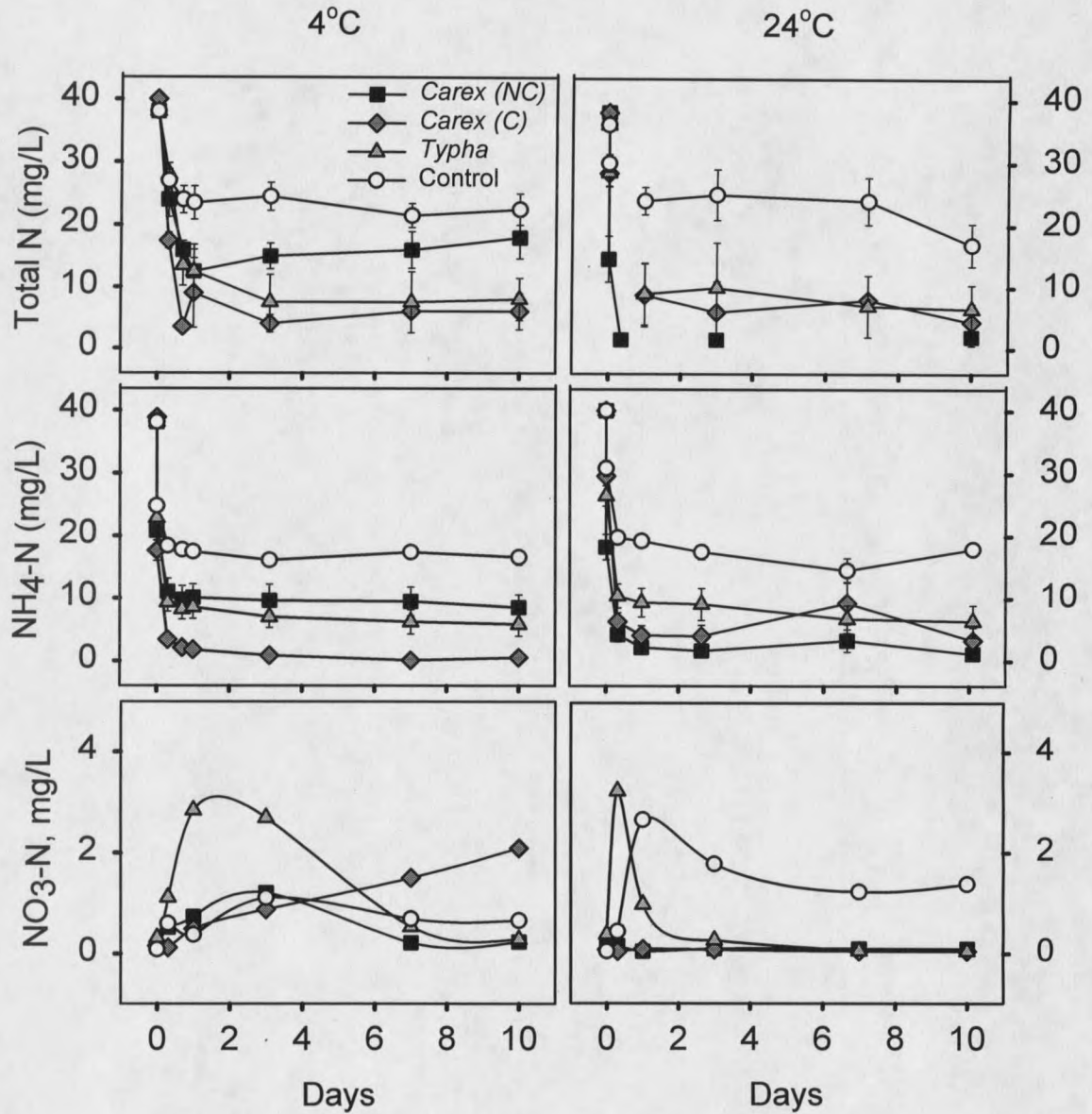


Figure 5. Total nitrogen and ammonium-N arithmetic means $\pm$ one standard error, and nitrate-N geometric means. Values taken over replicates and incubations during winter (4°C) and summer (24°C) conditions.

Table 3. Arithmetic mean $\text{NH}_4^+\text{-N}$ and geometric mean $\text{NO}_3\text{-N}$ concentrations $\pm$ one standard error. Values taken over all replicates and incubations within a season. Suffix letters indicate the rank and relationship between treatments at a given temperature and time (i.e. 4°C, day 1) at ($p \leq 0.05$). Asterisks at a given time (i.e. day 0) indicate a seasonal effect for a given treatment (i.e. *Carex(C)*) ($p \leq 0.05$). Comparisons were based on all combinations simultaneously evaluated.

Plant Treatment		$\text{NH}_4^+\text{-N(mg/L)}$				$\text{NO}_3\text{-N(mg/L)}$			
Day 0 (Upon filling)		4°C		24°C		4°C		24°C	
<i>Carex rostrata (C)</i>		18.90 $\pm$ 1.75	a*	29.48 $\pm$ 1.59	b*	0.16 $\pm$ 0.09	a	0.08 $\pm$ 0.04	a
<i>Carex rostrata(NC)</i>		19.74 $\pm$ 1.99	a	18.34 $\pm$ 1.75	a	0.14 $\pm$ 0.08	a	0.14 $\pm$ 0.07	a
<i>Typha latifolia</i>		23.90 $\pm$ 1.66	a	26.54 $\pm$ 1.32	b	0.32 $\pm$ 0.16	a	0.38 $\pm$ 0.15	a
Unplanted control		23.81 $\pm$ 2.15	a	30.92 $\pm$ 1.32	b	0.09 $\pm$ 0.06	a	0.05 $\pm$ 0.02	a
Day 0.3		4°C		24°C		4°C		24°C	
<i>Carex rostrata (C)</i>		3.43 $\pm$ 1.73	a	11.78 $\pm$ 4.88	ab	0.12 $\pm$ 0.08	a	0.05 $\pm$ 0.10	ab
<i>Carex rostrata(NC)</i>		11.06 $\pm$ 1.84	b	4.29 $\pm$ 1.63	a	1.12 $\pm$ 0.6	a	0.16 $\pm$ 0.10	ab
<i>Typha latifolia</i>		12.82 $\pm$ 1.54	b	9.77 $\pm$ 1.73	a	0.52 $\pm$ 0.38	a	3.24 $\pm$ 2.19	a
Unplanted control		17.84 $\pm$ 1.99	b	18.73 $\pm$ 1.73	b	0.60 $\pm$ 0.47	a	0.44 $\pm$ 0.30	b
Day 1		4°C		24°C		4°C		24°C	
<i>Carex rostrata (C)</i>		1.83 $\pm$ 1.92	a	4.65 $\pm$ 1.64	a	0.49 $\pm$ 0.30	a	0.09 $\pm$ 0.05	b
<i>Carex rostrata(NC)</i>		10.11 $\pm$ 1.92	bc	2.25 $\pm$ 1.81	a	0.74 $\pm$ 0.44	a*	0.05 $\pm$ 0.03	b*
<i>Typha latifolia</i>		9.04 $\pm$ 1.92	ab	9.41 $\pm$ 1.36	a	2.85 $\pm$ 1.72	a	0.98 $\pm$ 0.42	a
Unplanted control		17.47 $\pm$ 1.92	c	19.33 $\pm$ 1.36	b	0.38 $\pm$ 0.23	a	2.67 $\pm$ 1.14	a
Overall Day >1 ($\text{NH}_4\text{-N}$)									
Day 10 ($\text{NO}_3\text{-N}$)		4°C		24°C		4°C		24°C	
<i>Carex rostrata (C)</i>		0.84 $\pm$ 1.07	a*	5.22 $\pm$ 0.92	ab*	2.09 $\pm$ 1.29	a	0.05 $\pm$ 0.03	b
<i>Carex rostrata(NC)</i>		9.44 $\pm$ 1.07	b	2.13 $\pm$ 1.01	a	0.23 $\pm$ 0.15	a	0.10 $\pm$ 0.06	b
<i>Typha latifolia</i>		7.57 $\pm$ 1.07	b	7.93 $\pm$ 0.77	b	0.28 $\pm$ 0.18	a	0.05 $\pm$ 0.02	b
Unplanted control		16.92 $\pm$ 1.09	c	17.33 $\pm$ 0.76	c	0.66 $\pm$ 0.40	a	1.38 $\pm$ 0.60	a

to 0.12 ± 0.02 at 24°C , but with ($p=0.06$). *Typha* and the unplanted controls did not differ seasonally, with mean removal ratios of 0.19 ± 0.03 and 0.42 ± 0.02 , respectively.

Ammonium removal was positively influenced by the presence of plants during both seasons. A plant species effect was evident during the summer, when *Carex* outperformed *Typha*. A carbon treatment effect was shown only at 4°C , when *Carex* (C) attained higher removal than *Carex* (NC).

Ammonium Removal Prior to Day 1

Ammonium removal prior to day 1 established the overall treatment trends (Fig.6, Table 3). Temperature was a significant factor ($p= 0.004$) for removal at sampling time 0, with higher overall removal occurring at 4°C than at 24°C . Ammonium removal was generally higher for the planted treatments than the unplanted controls, and treatment differences became more distinct by 0.3 days. During 4°C , *Typha* and *Carex*(NC) performed similarly, but removed significantly less ammonium than *Carex* (C) at this time.

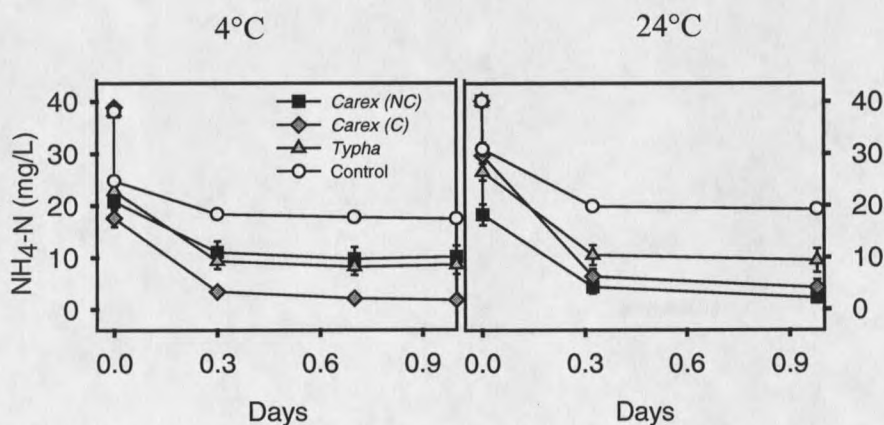


Figure 6. NH₄-N means $\pm$ one standard error prior to day 1. Values taken over all replicates and incubations during winter (4°C) and summer conditions (24°C).

Ammonium Sorption

The series of bottle extraction experiments performed on sterilized column gravel indicated that sorption was a curvilinear and immediate reaction. Within 12 hours, $69\% \pm 5\%$ of applied 40 mg/L and 5 mg/L ammonium was removed from the aqueous solution (Fig.7a). Equilibrium conditions occurred by 2 days. This time to equilibrium was used to obtain the maximum sorptive capacity of the gravel (Fig.7b). The sorptive capacity was several orders of magnitude beyond the influent $\text{NH}_4^+\text{-N}$ applied in the columns (40mg/L), and was not reached at 6000mg/L of applied ammonium.

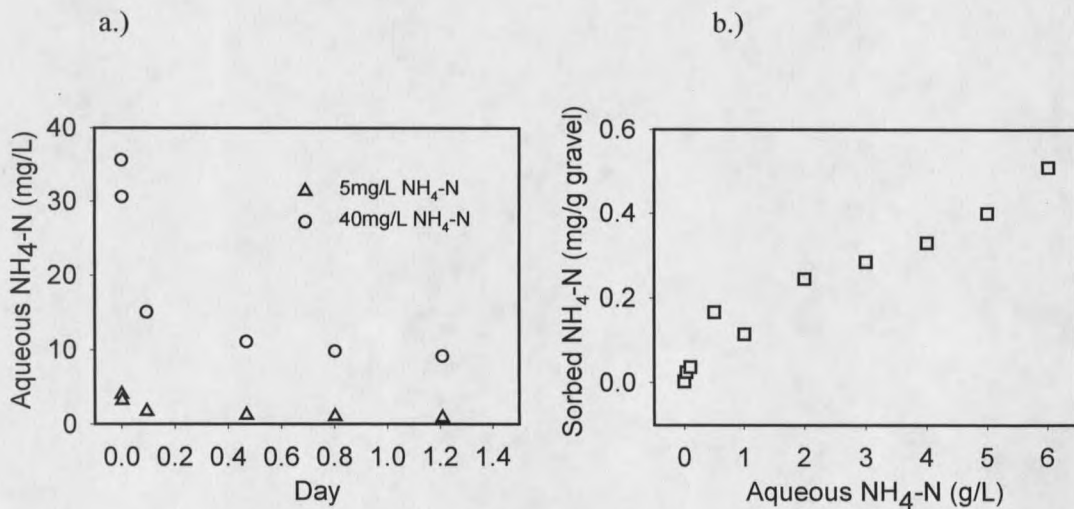


Figure 7. Mean results from bottle sorption experiments performed on triplicate subsamples of sterilized, wetland gravel. Figure a) equilibrium curves for high (40ppm) and low (5ppm) $\text{NH}_4\text{-N}$ application, and b) potential sorption capacity taken at 2 days.

Nitrate Production and Consumption

Nitrate was detected in the aqueous phase through both seasons, and either occurred at moderate (2-5mg/L) to high (20-30mg/L) levels, or was zero within an incubation. However, columns of the same treatment did not necessarily respond similarly for a given incubation, nor did individual columns perform the same between incubations of the same season. *Carex* columns showed particularly high variability.

Though an inclusive model with a time regression could not be developed due to the non-constant slopes, the treatment comparisons were similar between days (Fig.5, Table 3). During winter conditions, no wastewater or plant treatment differences were statistically significant. During the latter days of the summer cycle, the planted treatments exhibited similar means, but were significantly lower than the controls. Seasonal effects within treatments were generally not observed. However, temperature was an important predictor over all treatments ($p < 0.05$), and higher NO_3^- -N values occurred in the aqueous phase during 4°C than 24°C.

The nitrate profiles in time were also distinctly seasonal. During the winter at 4°C, the *Typha*, *Carex* (NC), and controls displayed net nitrate production from 0 to 3 days, then net consumption to near zero levels by day 10. Conversely, the *Carex*(C) treatment had continuously increasing NO_3^- -N concentrations throughout the winter cycle. At 24°C, the production and consumption of nitrate in the *Typha* and control columns occurred more rapidly. The two *Carex* treatments displayed nitrate levels near zero throughout the summer incubations.

Sulfate Production

Though sulfate was not added to the columns, reduced sulfur compounds were present in the sediment from previous application. As with nitrate, sulfate levels in the aqueous solution were significantly higher during the winter (4°C) season (Fig. 8), when all four treatments performed similarly. During summer conditions, the controls exhibited higher sulfate levels than the planted treatments.

Despite the similar trends between sulfate and nitrate, there was not a statistical correlation in the production of these anions. No measurable correlations between total organic carbon levels and sulfate or nitrate production were evident.

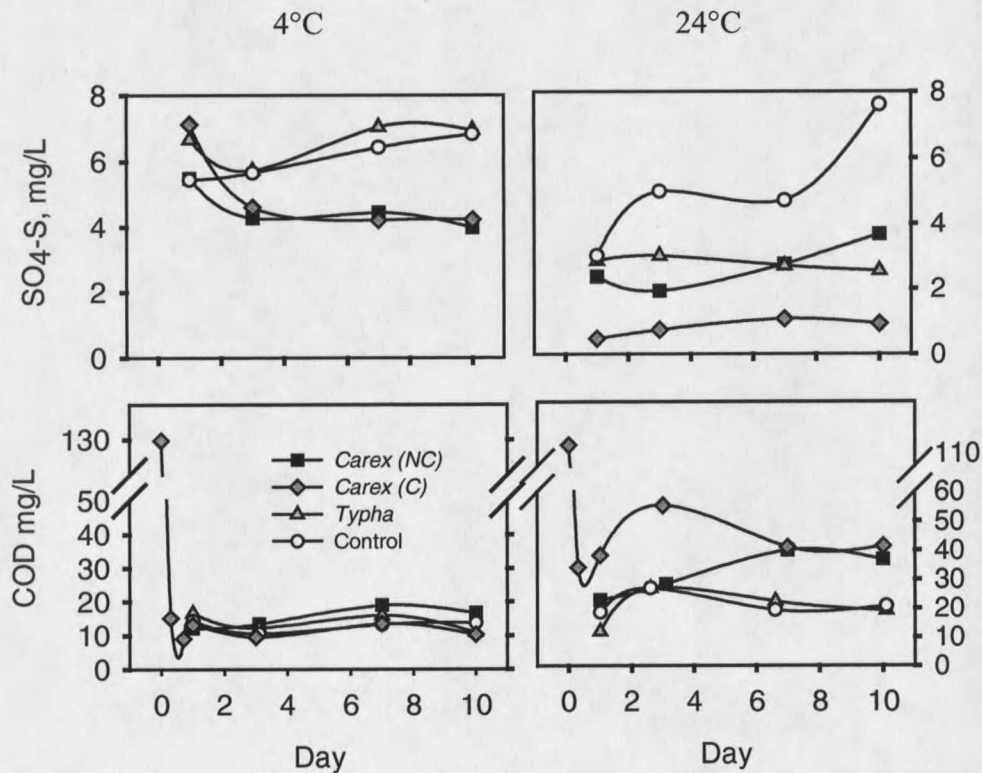


Figure 8. Mean sulfate-S and COD concentrations. Values taken over all replicates and incubations during winter (4°C) and summer (24°C) conditions.

COD Production and Consumption

Due to degradation in the mixing tank, the COD influent was particularly variable, and ranged from 67-200 mg/L COD. This variability did not statistically compromise the effects of the treatment *Carex* (C) in the ammonium model, and was accounted for within carbon, and carbon-nitrogen interaction analyses. Though carbon levels were best represented by total organic carbon measurements, sufficient data was not available. The TOC/COD ratios did not significantly differ among treatments after 24 hours (Table 4); therefore, COD was considered a valid scale for carbon.

In the COD degradation plots (Fig.8, Table 4), the amended *Carex* (C) exhibited significantly higher levels of COD removal during 4°C ($C/C_0 = 0.09 \pm 0.03$) than at 24°C ($C/C_0 = 0.44 \pm 0.02$). By day 7, the remaining COD of *Carex* (C) was similar to *Carex* (NC). In the unamended columns, higher levels of internally-produced COD were evident during the summer. *Carex* (NC) exhibited the highest COD mean during the summer (32 ± 2 mg/L), versus the *Typha* and controls, which had comparable values of 21 ± 1 mg/L COD. Incubation day was not a significant predictor in the behavior of COD behavior in the amended or unamended columns.

Table 4. Mean COD concentrations and COD/TOC ratios $\pm$ one standard error. Values taken over replicates and incubations within a season. Suffix letters indicate the rank and relationship between the unamended treatments at a given temperature and time (i.e. 4°C, day 1) at ($p \leq 0.05$). Asterisks at a given time (i.e. day 0) indicate a seasonal effect for a given treatment (i.e. *Carex(C)*) ($p \leq 0.05$). Comparisons were based on all combinations of unamended treatments, simultaneously evaluated.

Plant Treatment					
Day 0 (Upon filling)	4°C		24°C		COD/TOC
<i>Carex rostrata (C)</i>	118 $\pm$ 21.6	--	148 $\pm$ 17.2	--	3.92 $\pm$ 0.81
<i>Carex rostrata(NC)</i>	31.1 $\pm$ 6.39	a	19.0 $\pm$ 5.63	a	
<i>Typha latifolia</i>	14.2 $\pm$ 5.63	a	21.6 $\pm$ 4.22	a	
Unplanted control	10.2 $\pm$ 7.56	a	14.3 $\pm$ 4.22	a	
Day 1	4°C		24°C		
<i>Carex rostrata (C)</i>	13.13 $\pm$ 5.76	*	35.1 $\pm$ 4.91	*	
<i>Carex rostrata(NC)</i>	15.75 $\pm$ 3.04	a	22.8 $\pm$ 2.87	a	
<i>Typha latifolia</i>	16.62 $\pm$ 3.04	a	11.9 $\pm$ 2.15	a	
Unplanted control	14.25 $\pm$ 3.04	a	18.5 $\pm$ 2.15	a	
Overall Day > 1	4°C		24°C		
<i>Carex rostrata (C)</i>	11.3 $\pm$ 2.65	*	41.8 $\pm$ 2.14	*	2.14 $\pm$ 0.20
<i>Carex rostrata(NC)</i>	16.8 $\pm$ 2.14	a	32.0 $\pm$ 1.85	a	2.98 $\pm$ 0.31
<i>Typha latifolia</i>	13.7 $\pm$ 2.10	a	20.0 $\pm$ 1.40	a	2.76 $\pm$ 0.29
Unplanted control	13.0 $\pm$ 2.14	a	21.3 $\pm$ 1.40	a	3.24 $\pm$ 0.28

Carbon and Nitrogen Interaction

The association between ammonium removal and COD loads was continuous, and dependent on season (Fig. 9). During the winter at 4°C, increased COD loads occurred with an increase in $\text{NH}_4\text{-N}$ removal performance. Conversely, influent COD levels negatively affected ammonium removal during the summer at 24°C.

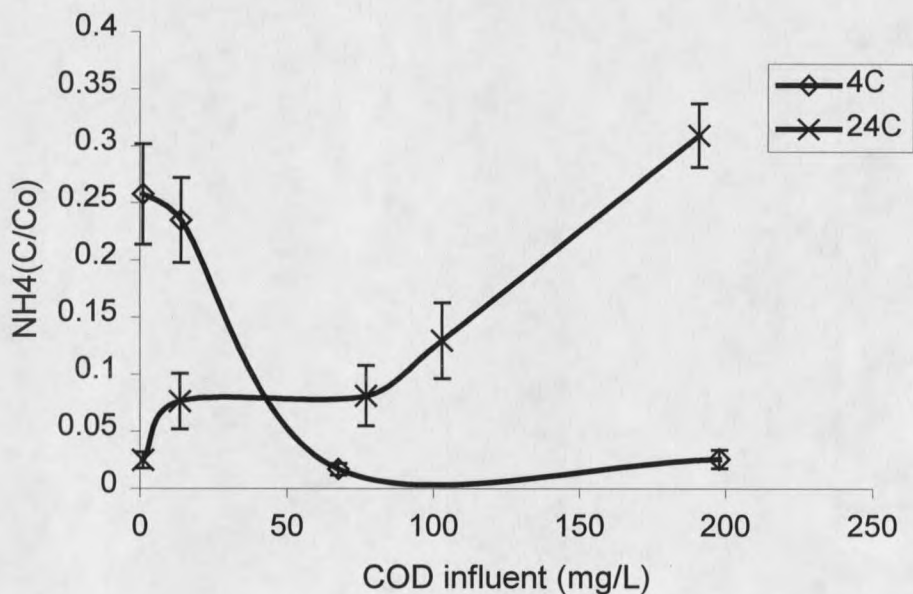


Figure 9. $\text{NH}_4\text{-N}$ and COD interaction plot at 4 and 24°C based on *Carex*. $\text{NH}_4\text{-N}$ removal means $\pm$ one standard error over days and replicates, for a given COD influent value.

Nitrogen Isotope Tracer Experiment

The nitrogen isotope tracer experiments were performed to further quantify nitrogen removal pathways. N^{15} was added as 50% of the influent ammonium concentration, and the columns were destructively sampled at the end of the 10-day residence time. The results from the winter incubation were inconclusive due to analytical difficulties, but results from the summer destructive sampling of a *Carex* (NC), *Carex* (C), and unplanted control are presented.

Four layers were discernable in the planted column profiles (Figure 10). The top 1 cm consisted of a dark layer of organic matter. The majority of *Carex* roots and rhizomes reached a depth of approximately 20cm. The basal roots constituted a small portion of the total root mass; adventitious roots and rhizomes formed a tight, thick mat



Figure 10. Destructively sampled *Carex* column during the summer N^{15} study.

that primarily lined the circumference of the column. The interstitial gravel was relatively devoid of visible biofilms and particulate matter. However, a layer of muck and silt was deposited 5 cm from the bottom of each column, likely due to the draining procedure.

The size of the aqueous, gravel, and plant compartments showed high variability among columns (Table 5). The planted columns contained less gravel and less porewater than the controls. Though the two *Carex* columns of the summer incubation were experimentally similar units, one showed eight times the plant matter of the other. Consequently, the comparative statistical analysis was performed on the recovered N^{15} on a unit mass basis for plant and gravel matter, and unit volume basis for the aqueous phase (Table 6).

The N^{15} recovery profile on day 10 indicated both a carbon and planted treatment effect (Fig.11). The difference between *Carex*(C) and *Carex*(NC) was shown in the assimilated and "unaccounted" pools. *Carex*(NC) assimilated $58\% \pm 4\%$ of applied N^{15} into below and above-ground plant matter, in contrast to the *Carex* (C) which assimilated $5\% \pm 0.5\%$ N^{15} . Though the plant density of the unamended *Carex* was greater than *Carex* (C), the total N^{15} uptake on a unit mass basis was also significantly higher. Plant uptake was the most predominant removal mechanism in the unamended *Carex*. The unaccountable N^{15} was 67% of applied values in *Carex* (C) verses 19% of N^{15} in the unamended *Carex*. The "unaccounted" partition included organic nitrogen on the gravel and in the drained wastewater, and denitrified nitrogen gases. Experimental error may also be included in this portion.

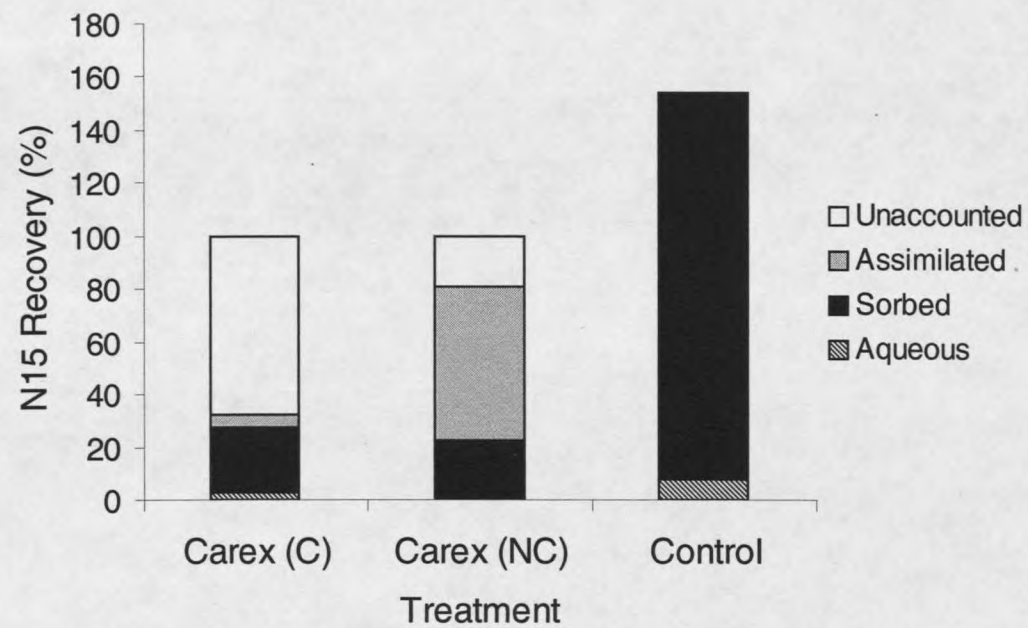


Figure 11. N¹⁵ recovery profile from destroyed *Carex* (C), *Carex* (NC), and unplanted control during the summer incubation.

Table 5. Destructive sampling partitions. Plant matter and gravel measured as dry weight (g), and aqueous phase as volume (mL). AG= above-ground, BG=below-ground.

Parameter	<i>Carex</i> (NC)	<i>Carex</i> (C)	<i>Carex</i> (NC)	Control
Season	Winter	Summer	Summer	Summer
Column Number	20	22	23	24
Plant matter-AG (g)	94	50	160	
Plant matter-BG (g)	206	43	561	
Gravel (g)	19092	22369	21684	24412
Aqueous solution (ml)	3698	5000	4700	3800

Table 6. Summer N¹⁵ recovery in percentage or unit mass (μg/g) or volume (μg/ml) basis. AG=above-ground, BG=below-ground, ND = not detected.

Partition	Percent Recovery			Recovered Mass N ¹⁵ /unit mass,vol (μg/g,ml)			
	<i>Carex</i> (C)	<i>Carex</i> (NC)	Control	<i>Carex</i> (C)	<i>Carex</i>	Control	
Aqueous-NH ₄ -N	3.04	0.19	4.03	0.45	0.03	0.59	(μg/ml)
Aqueous-NO ₃ -N	ND	ND	3.44	ND	ND	0.51	(μg/ml)
Sorbed-BG.plant	0.11 ± 0.03	2.49 ± 0.83		1.94 ± 0.44	3.27 ± 1.09		(μg/g)
Sorbed-Gravel	24.0 ± 2.84	20.2 ± 1.00	147 ± 21.3	0.79 ± 0.09	0.69 ± 0.03	3.37 ± 0.49	(μg/g)
Assimilated-AG.plant	1.35 ± 0.27	20.4 ± 1.06		19.9 ± 4.78	94.1 ± 5.98		(μg/g)
Assimilated-BG.plant	3.74 ± 0.25	37.9 ± 3.21		63.6 ± 4.31	49.8 ± 4.21		(μg/g)
Assimilated-Total	5.09	58.4		83.5	144		(μg/g)
Unaccounted	67.4	18.8					

The *Carex* (C) and control columns displayed similarly high levels of aqueous ammonium-N¹⁵, which were 3% and 4% of the respective mass balances. The unamended *Carex* exhibited 0.2% of recovered N¹⁵ in the aqueous phase. Aqueous nitrate was undetected in the planted columns, but contributed 3% of the applied N¹⁵ in the control at day 10.

Sorption was a considerable mechanism for ammonium removal in all columns. In the carbon and non-carbon *Carex* treatments, $24\% \pm 3\%$ and $20 \pm 1\%$ N¹⁵ was recovered on the gravel, respectively. Plant roots also provided substantial surface sites. In the planted columns, the total sorbed ammonium represented the second highest mass fraction. Sorption was the principal removal mechanism in the unplanted control column, and represented $146\% \pm 21\%$ recovered N¹⁵. This unreasonably high value demonstrates the error that was potentially incurred through scaling the sub-samples to the total mass or volume components of the columns. Though sub-samples were tested in triplicate, caution must also be used in the interpretation of results due to the lack of column replicates.

CHAPTER 4

DISCUSSION

Though several separate experiments and parameters were included in this study, they will be shown to collectively support the following conclusions:

- I. Due to an increased availability of oxygen, nitrogen removal was not significantly hindered by plant dormancy and cold-temperatures.
- II. The effect of a carbon amendment on nitrogen removal was seasonally dependent, and caused an apparent shift in nitrogen removal pathways.
- III. Despite the biotic changes that occurred in response to carbon load, oxygen availability, and season, a sufficient level of nitrogen removal was maintained. This stability was largely attributed to the abiotic effects of sorption.

The experimental results are discussed within the thematic context of these three points.

Seasonal Oxygen Availability

The results from the individual carbon and nitrogen models suggest that the removal of these parameters was not hindered by cold-temperature, dormant conditions. *Carex (C)* removed higher NH_4^+ -N than *Carex (NC)* at 4°C. *Carex (C)* also exhibited higher COD removal during the winter. The carbon-nitrogen interaction model also implied that the co-presence of carbon did not inhibit, and even encouraged nitrogen removal during this time.

The implicating factor in these results is the increased availability of oxygen in the rhizosphere during the winter versus summer. Higher levels of oxidized nutrients like nitrate and sulfate were displayed at 4°C. Results from the previous experiment (Allen 1999) also exhibited higher sulfate and redox values in the planted treatments during the winter at 4°C.

Oxygenation of the rhizosphere is promoted during the dormant, winter conditions because plants are not actively respiring (Gries et.al 1989; Callaway and King 1996). Atmospheric oxygen may also be transported via convective or diffusive flow through dead cattail and reed culms (Brix *et al.* 1996). The increased surface area that below-ground plant matter provides may also encourage oxygen retention during the draining of the column.

The influence of exogenous carbon in the wastewater may be especially significant during the summer, when the electron acceptor is limiting, as indicated by the carbon-nitrogen interaction model. In addition, higher levels of internally-produced carbon occur during the summer, as shown in the unamended columns. This internal

carbon may increase the oxygen demand and lower the apparent COD removal performance. If this carbon is predominantly a result of decomposition processes, as literature implies, then an analogous production of nitrogen could also potentially affect ammonium removal during the summer.

Treatment and Seasonal Effects on Nitrogen Removal Mechanisms

Increased levels of electron acceptors during winter conditions evidently countered any negative effects of cold temperatures, like decreased metabolic rates. Though heterotrophic activity is plausible under conditions as cold as 4°C, nitrifiers are severely inhibited under these conditions (Sharma and Ahler 1977). Nitrifiers were evident during this time; higher overall nitrate values occurred at 4°C versus 24°C. However, the significance of coupled nitrification/denitrification is questionable during both seasons. The geometric means of aqueous $\text{NO}_3\text{-N}$ in the columns never rose above 3 mg/L. In the previous experiment under high-carbon conditions (Allen 1999), no aqueous nitrate was detected, despite the high levels of $\text{NH}_4^+\text{-N}$ removal.

The possibility of simultaneous production and consumption of nitrate limits the conclusions that can be made regarding the strength of the nitrification process. The observed concentrations must be viewed as a function of the nitrification and denitrification potential of the surface films, and the subsequent diffusion of $\text{NO}_3\text{-N}$ into the measured phase. The change in $\text{NO}_3^-\text{-N}$ production and $\text{NH}_4^+\text{-N}$ consumption per column were uncorrelated over a time step, dt . Though this lack of a correlation does not disprove the significance of gross nitrification, it does suggest that the coupled

nitrification/denitrification and diffusion on a column to column basis was highly irregular. Nitrogen removal was not dependent upon the strength of this coupled process.

During the winter when plants are not assimilating nutrients, immobilization and sorption are the remaining nitrogen removal mechanisms. The positive relationship between carbon and nitrogen is likely due to immobilization. The increased COD removal (Fig.8) during winter versus summer corresponds to the seasonal oxygen availability. The rapidity of heterotrophic kinetics, and their competitiveness for available oxygen from draining and filling, is shown in the immediate degradation of COD in the amended columns (Fig.9), and corresponds to the increased nitrogen removal of *Carex* (C) versus *Carex* (NC) identified prior to day 1.

The higher "unaccountable" fraction in the carbon-amended *Carex* of the N^{15} isotope study may also be attributed to immobilization. The depletion of 150mg/L of COD, and the implicit assimilation of nitrogen, occurred in the traced *Carex* (C) within 24 hours. Unless sufficient oxygen was available after the growth and subsequent decay of this biomass, nitrification would be limited. Nitrate was not detected in either the *Carex* (C) and *Carex* (NC) during the tracer experiment. Under the increased electron donor load, it is unlikely that the oxygenation potential of *Carex* (C) was significantly greater than *Carex* (NC). Hence, the higher unaccountable levels shown in *Carex* (C) are not likely due to coupled nitrification and denitrification.

Though the microbial biomass associated with the roots was included in the assimilated plant N^{15} digestion, it is likely that a portion of the unaccounted nitrogen accumulated on the gravel, as shown in other mass balance studies, or washed out upon

draining. An increasing number of studies suggest that vegetated subsurface-flow wetlands operate as anaerobic fixed-film reactors that may entrap organic matter within biofilms (EPA 2000). In Reddy *et al.* (1989), organic fractions within the loam substratum were measured at 11% and 17% ^{15}N in the planted and unplanted treatments, respectively. Breen (1990) found approximately 10% applied ^{15}N immobilized within gravel biofilm, and 5% within organic films on the roots.

The carbon amendment, and implicit stimulation of heterotrophic bacteria also affected the relative strength of plant assimilation (Fig.11). The unamended *Carex* exhibited higher total plant uptake per unit mass. This result was also shown by Zhu and Sikora (1995) for a range of wetland plant species loaded with $\text{NO}_3^- \text{N}$. Plant uptake was the predominant removal mechanism for columns without exogenous carbon, in contrast to the units with carbon, which exhibited high "unaccountable" levels. This unknown fraction was attributed to combined immobilization and denitrification. When stimulated by a labile carbon source, denitrifiers and other heterotrophs may be better competitors than plants for nitrogen (Schimel *et al.* 1989), or decreased plant respiration and nutrient uptake may be a direct function of lower redox levels initiated by the carbon load (Morris and Dacey 1984).

However, the practical application of the interdependence between plants and heterotrophs may be small. Enough data from other experiments has ascertained that plant uptake is not an important nitrogen removal mechanism under typical wastewater nitrogen and carbon loads and stand establishment (Shaver and Melillo 1984; Tanner 1996; EPA 2000). Though *Carex* attained higher $\text{NH}_4\text{-N}$ removal than *Typha* at 24°C ,

these differences are likely due to the age of the column mesocosms. The EPA (2000) suggests that plant selection be based on native, regional species.

Stability of Nitrogen Removal Performance

Sorption was shown to largely control the immediate decline of aqueous ammonium in the column mesocosms. Similar results have also been found in gravel systems by Sikora *et al.* (1995). As indicated by the N^{15} study, sorption onto organic and inorganic substrata represented a large sink for aqueous ammonium – even at the end of the 10-day incubation period, during the third year of operation. Sorption remained influential even in the presence of other mechanisms, like plant and microbial uptake. The importance of this process is likely unique to drain-and-fill, batch systems, where the regeneration of surface sites is encouraged. Sorption is not widely shown in full-scale, continuous flow wetlands, except during periods of system start-up (Brix 1987).

The fundamental attribute of wetland macrophytes may be their enhancement to the below-ground structure, in terms of surface area and biochemical microniches. The immediate effects (day < 1) shown in the planted treatments, especially *Carex*, on winter and summer nitrogen removal support this argument, since the kinetics of sorption – a mechanism dependent upon surface area – controlled during that time. Though the presence of plants had a positive effect on nitrogen removal during both seasons in the column mesocosms, they did not significantly improve performance in a bench-scale, continuous-flow system that has operated under similar conditions at Montana State University (Biederman 1999). The continuous-flow system did not exhibit the immediate

effects attributable to sorption, which may partially explain the insignificant treatment effect of plants.

Carex rostrata has repeatedly shown high levels of oxygenation capability and nutrient removal (Allen 1999). A potential mechanism for the higher performance in *Carex* may be structural; sedges and grasses exhibit an intricate rhizosphere that may increase the available pore space for oxygen diffusion, especially during the drain-and-fill regime. Other wetland plants that have been identified in promoting nutrient transformation through root-zone oxidation include: pennywort, waterhyacinth, pickerelweed, and arrowhead (Reddy *et al.* 1990). In particular, pennywort (*Hydrocotyle umbellata*) has shown high oxygenation capabilities, and also exhibits a fine root structure (Moorhead and Reddy 1998).

The stability of the sorption process may substantiate the similar behavior for carbon and nitrogen removal between years, season, and wastewater load. The large sorptive capacity of the gravel and plant material, and its importance during the third year of operation, suggest that this mechanism may indeterminately remain a significant process. Sorption indirectly promotes microbial utilization by accumulating nutrients at the surface and controls subsequent reactions. If sorbed NH_4^+ -N becomes oxidized to NO_3^- -N during draining, sorption may also facilitate the permanent removal of nitrogen in this manner. McBride and Tanner (2000) showed that NH_4^+ -N removal increased with column draining frequency, and that sorbed NH_4^+ -N was a partial source of produced NO_3^- -N.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Results from this experiment indicate that high levels of nitrogen removal were obtained during both winter and summer conditions, and were not markedly affected by carbon load. Total nitrogen removal ranged from 62 to 94% in the planted columns, and 41% in the unplanted controls. The presence of plants was contributive to exogenous carbon and nitrogen removal during both seasons, and *Carex* attained higher nitrogen removal than *Typha* during the summer season.

Though this system achieved high levels of treatment, interpretations must incorporate the system effects of the column reactors, like intermittent oxidation of the substratum, batch conditions, and a high plant surface area per wetland volume. The seasonal conditions did not include other environmental factors like snow and ice, which could also limit performance. Full-scale, continuous flow wetlands have typically shown inadequate levels of nitrogen removal, especially during the winter season.

However, the results from the column mesocosms may provide insight into the poor performance shown in other wetlands. Compared to other systems, these columns optimize oxidation of the substratum, and this experiment also removed the effect of a preferential electron donor. The coupled nitrification/denitrification process was an erratic, uncorrelated influence on nitrogen removal. Nitrification/denitrification is expected to be even less effective in full-scale systems. The latest EPA manual (2000)

has re-evaluated earlier assumptions based on the availability of oxygen and associated nutrient transformations, and has defined even the root-zone as anaerobic.

Though plant assimilation represented a significant pool in the tracer study, plants do not provide a realistic nitrogen removal mechanism. Even if the seasonal plant assimilation of established stands was significant, harvesting plant biomass is inefficient and costly. Despite the chemical and structural amendments that plants provide all year, this study indicated that macrophytes limit oxygen availability in the summer due to respiration and internal nutrient production. Though high-biomass, aerenchymous plants like *Typha latifolia* have been endorsed in wetland literature, grasses and sedges like *Carex rostrata* may exhibit less oxygen demand and a superior root environment.

In this experiment, nitrogen removal was predominantly controlled by sorption and immobilization. The relatively young age, hydraulic conditions, and substratum characteristics of the columns may have emphasized the storage capabilities for nitrogen. These processes are likely finite in full-scale systems, hence the insufficient nitrogen removal shown in most wetlands. The permanent removal of nitrogen is limited in constructed wetlands, and should be mediated through other designs, like open water areas. Operating full-scale systems in batch, or with periodic draining, may initiate similar mechanisms and removal capacities of this experiment. The pretreatment of a high-carbon wastewater is likely ineffectual; nitrifying bacteria are restricted foremost by the lack of oxygen in wetland environments.

Though specific mechanisms are becoming better defined, the lack of valid performance data, and the inconsistency between extant models and empirical data

inhibits wetland design. The format of models should be reviewed, and reflect the state of knowledge that has been obtained. Then, empirical methods may be better directed.

Wetland science may also advance with techniques like in-situ probing and biofilm analyses. The continuation of the North American Treatment Wetlands database is essential, and should be more accessible to scientists, not associated with the EPA, to contribute and obtain information.

In qualitative terms, wetlands must also be recognized as a limited energy source in the form of decay and assimilative homeostasis. Wetlands provide a valuable source of information regarding the biological degradation process, and may also functionally render the sustainable treatment of our waste, if the conditions and limitations are appropriately acknowledged.

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