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Transformation and fate of nitrate in northern prairie wetlands

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Transformation and fate of nitrate in northern prairie wetlands

by

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TABLE OF CONTENTS

GENERAL INTRODUCTION

Background

The recognition of wetlands as ecologically valuable landscape components has occurred only within the last two decades. In a review of research on the role of freshwater wetlands and water quality, Nixon and Lee (1986) note that "much of the effort during this time has been confined to studies of productivity, habitat value, and other aspects of wetlands that do not necessarily yield the kinds of information necessary to evaluate the links between wetlands and adjacent waters in terms of nutrients, heavy metals, or other pollutants." They emphasized this point because "some of those charged with constructing, managing, or regulating the uses of these environments may find themselves discouraged on learning that the evidence necessary to resolve such a basic question as the role of wetlands in water quality is so often preliminary, incomplete, flawed, of lacking."

Much of the current interest in the role of wetlands in providing water quality functions can be traced to the practice of using wetlands for small-scale sewage or runoff treatment (Kadlec and Tilton 1979, Whigham 1982). The primary assumption of this practice is that wetlands are sinks in the biogeochemical cycles of carbon, nutrients, heavy metals, and various other pollutants. However, in the case of nitrogen and phosphorus, Nixon and Lee (1986) state that "while wetlands appear to serve as sinks for these elements, the amount of these materials retained varies widely and does not appear to correlate in any simple way with inputs." These authors state that "we need to learn more before the scientific community is in a position to make a credible quantitative assessment of the potential role of wetlands in water quality improvement."

The recognition of the water quality functions and values of wetlands has recently led to increasing focus on the utilization of restored or created wetlands as nutrient sinks for non-point source pollution in agricultural landscapes. Based on both wetland drainage and surface water quality criteria, one of the regions where the restoration of wetlands may result in significant water quality improvements is the Midwestern corn belt (van der Valk and Jolly 1992). In Iowa, for example, 99% of the native wetlands have been drained and over 90% of the total land area is used for agricultural production. Nitrate (NO_3^-) is one of the agricultural chemical contaminants of foremost concern in the Midwestern corn belt

because of its potential impact on public health and ecosystem function, and because of the widespread use of nitrogen in modern agriculture. Non-point loads of inorganic nitrogen to surface waters in the region are among the highest in the country (Omernik 1977) and nitrate concentrations have continued to increase in many surface waters (Hallberg 1989).

If wetlands are to serve as long term sinks for nitrogen, differences in inputs and outputs must reflect net storage in the system through accumulation and burial in the sediments, or net loss from the system through gaseous evolution of NH_3, N_2O, N_2. Denitrification is the process whereby nitrate is reduced by facultatively anaerobic bacteria to nitrous oxides or dinitrogen gas. The reaction occurs under anoxic conditions (Eh = +350 to +100 mV), where nitrate is used in place of oxygen as the terminal electron acceptor during the oxidation of organic matter (Tiedje 1988). Several reviews have addressed the biochemistry and physiology of denitrification (Painter 1970, Payne 1973, Focht and Verstraete 1977, Knowles 1982, Tiedje 1988), and the rates of denitrification in marine (Knowles 1982, Hattori 1983), stream, river, lake and subtidal coastal marine ecosystems (Seitzinger 1988, 1990).

Most of the published papers dealing with freshwater wetlands and water quality note the probable importance of denitrification. In fact, with rare exception, denitrification is cited as the primary reason wetlands may serve as nitrogen sinks (Lee et al. 1975, van der Valk et al. 1979, Davis et al. 1981, Gersberg et al. 1983). However, there have been few measurements of denitrification in freshwater marshes (Howard-Williams 1985, Nixon and Lee 1986, Bowden 1987, Seitzinger 1988, Neely and Baker 1989). As Neely and Baker (1989) note, denitrification is only assumed to be an important process in many freshwater wetlands based largely on circumstantial evidence; first, that conditions in the wetlands are suitable for denitrification (anaerobic conditions and a large base of organic carbon) and, second, that nitrate disappears rapidly from water overlying wetland sediments. Research is needed to assess realistic nitrate transformation rates, to determine the fate of transformed nitrate, and to identify factors which affect the rates of nitrate transformation or limit freshwater wetlands in the sustained removal of externally loaded nitrate.

An explanation of the dissertation organization

The research that is presented in this dissertation addressed the transformation and fate of nitrate in northern prairie wetlands. The research utilized a combination of wetland mesocosms and microcosms to conduct controlled and replicated experiments involving nitrate transformations with the overall objectives: 1) to estimate the capacity of restored or natural northern prairie wetlands as sinks for externally loaded nitrate, 2) to determine the fate of transformed nitrate, and 3) to begin to identify the factors which limit the sustained abilities of northern prairie wetlands to act as sinks for nitrate.

The dissertation is divided into three sections which are manuscripts intended for publication and are followed by a General Summary. References cited in the General Introduction and General Summary follow the General Summary.

Paper I describes experiments investigating the transformation and fate of nitrate in northern prairie wetlands. Experimental wetland mesocosms were utilized to obtain realistic estimates of the rates of nitrate transformation, to conduct ^{15}N tracer studies designed to determine the fate of externally loaded nitrate, and to investigate the effects of nitrate loading pattern on transformation rates.

Paper II describes the results of experiments investigating the role of decaying plant litter in the transformation and fate of nitrate in northern prairie wetlands. First, to establish the presence of anaerobic microzones, oxygen distribution at sediment-water and litter-water interfaces was measured using a dissolved oxygen microelectrode. Secondly, the transformation and fate of nitrate in the presence and absence of litter was examined at two different scales. Enclosures placed *in situ* within wetland mesocosms were utilized to provide field scale estimates and sediment-water microcosms were utilized to allow greater experimental control and the use of ^{15}N tracers.

Paper III describes the results of ^{15}N tracer studies conducted in sediment-water microcosms containing intact sediment cores designed to determine the effects of nitrate concentration in the overlying water on nitrate flux. The intact sediment cores were collected from within the experimental mesocosms and from a recently restored northern prairie wetland.

PAPER I: TRANSFORMATION AND FATE OF NITRATE IN NORTHERN PRAIRIE WETLANDS

INTRODUCTION

A recognition of the water quality functions and values of wetlands has recently led to increasing focus on the utilization of restored or created wetlands as nutrient sinks for non-point source pollution in agricultural landscapes. Based on both wetland drainage and surface water quality criteria, one of the regions where the restoration of wetlands may result in significant water quality improvement is the Midwestern corn belt (van der Valk and Jolly 1992). In Iowa, for example, 99% of the native wetlands have been drained and over 90% of the total land area is used for agricultural production.

Nitrate (NO_3^-) is one of the agricultural chemical contaminants of foremost concern in the Midwestern corn belt because of its potential impact on public health and ecosystem function, and because of the widespread use of nitrogen in modern agriculture. Non-point loads of inorganic nitrogen to surface waters in the region are among the highest in the country (Omernik 1977) and nitrate concentrations have continued to increase in many surface waters (Hallberg 1989).

If wetlands are to serve as long term sinks for externally loaded nitrogen, differences in inputs and outputs must reflect net storage in the system through accumulation and burial in the sediments, or net loss from the system through gaseous evolution of NH_3, N_2O, or N_2. Reference to works reviewing the biogeochemistry of nitrogen in freshwater wetlands, however, reveals that the importance of these processes is not well documented (Howard-Williams 1985, Nixon and Lee 1986, Bowden 1987). The few studies of the cycling of nitrogen in northern prairie wetlands have generally demonstrated that natural and artificial wetlands can serve, at least on a seasonal basis, as nitrogen sinks (van der Valk et al. 1979, Davis et al. 1981).

Most of the published papers dealing with freshwater wetlands and water quality note the probable importance of denitrification (Lee et al. 1975, Kadlec 1979, van der Valk 1979, Davis et al. 1981, Gersberg et al. 1983). However, there have been few actual measurements of denitrification in freshwater marshes (Nixon and Lee 1986, Bowden 1987, Seitzinger 1988, Neely and Baker 1989). As Neely and Baker (1989) note, denitrification is only assumed to be an important process in many freshwater wetlands based largely on circumstantial evidence, first that conditions in the wetlands are suitable for denitrification (anaerobic conditions and a large base of organic carbon) and second that nitrate disappears rapidly from water overlying wetland sediments.

Increased nitrate loading to wetlands in agricultural watersheds might be expected to stimulate denitrification resulting from increases in activities and/or population densities of denitrifying bacteria. However, there have been few measurements of the effects of loading patterns or other factors which might affect the denitrification capacity of wetlands receiving nitrate loads.

If restored or created wetlands are to be utilized as nitrogen sinks in agricultural landscapes, we need to greatly increase our understanding of the transformation and fate of non-point source nitrate within these systems. Such information is necessary to make credible management recommendations in terms of site selection and design criteria and to estimate sustainable nitrate loading rates.

METHODS

Wetland mesocosms were used to conduct controlled and replicated experiments investigating the transformation and fate of nitrate in northern prairie wetlands. The mesocosms were utilized to obtain realistic, ecosystem level estimates of the rates of nitrate transformation and to investigate the effects of nitrate loading pattern on transformation rates and processes within the systems. ^{15}N tracer experiments were also conducted to determine the fate of the externally loaded nitrate and the effect of nitrate loading pattern on denitrification capacity.

Description of experimental wetland mesocosm facility

These studies were conducted at the Iowa State University Experimental Wetland Facility (Crumpton et al. *in press*). This facility consists of 48 wetland mesocosms which were completed in 1989 (Figures 1 and 2). The mesocosms were constructed using uv stabilized polyethylene tanks which are 3.35 m in diameter and 90 cm deep, thus providing for approximately 9 m^2 of wetland in each mesocosm (Figure 3). The tanks were filled to a depth of 60 cm with an Okoboji silty clay loam (cumulic haplaquoll), planted with cattail rhizomes (*Typha glauca* Godr.), and flooded. The soil used in the mesocosms was excavated from a recently restored wetland at Jim Ketelsen Greenwing Marsh east of Ames, IA.

A deep irrigation well supplies feedwater for the mesocosms. Chemical characteristics of the unmodified feedwater were analyzed by the Analytical Services Laboratory of the Department of Civil and Construction Engineering, Iowa State University. The concentrations of anions and cations in the feedwater are similar to those found in wetlands in glaciated terrain (LaBaugh 1989) yet the concentrations of nitrogen and phosphorus are low enough to allow for experimental addition of these two elements (Table 1). For the research described here, the mesocosms were configured with in-line fertilizer injectors which allowed for the controlled addition of desired chemicals directly into the irrigation water. Mesocosms are individually valved and water is supplied to each unit through spray nozzles around its inside circumference. Bulkhead adapters for surface drainage are located 5 cm above the sediment to prevent loss of all water in the event of a leak. Water level is maintained through the use of variable height standpipes.

Figure 1. Aerial view of experimental wetland mesocosm complex. One cm equals 7.5 m

Figure 2. Ground level view of experimental wetland mesocosm complex

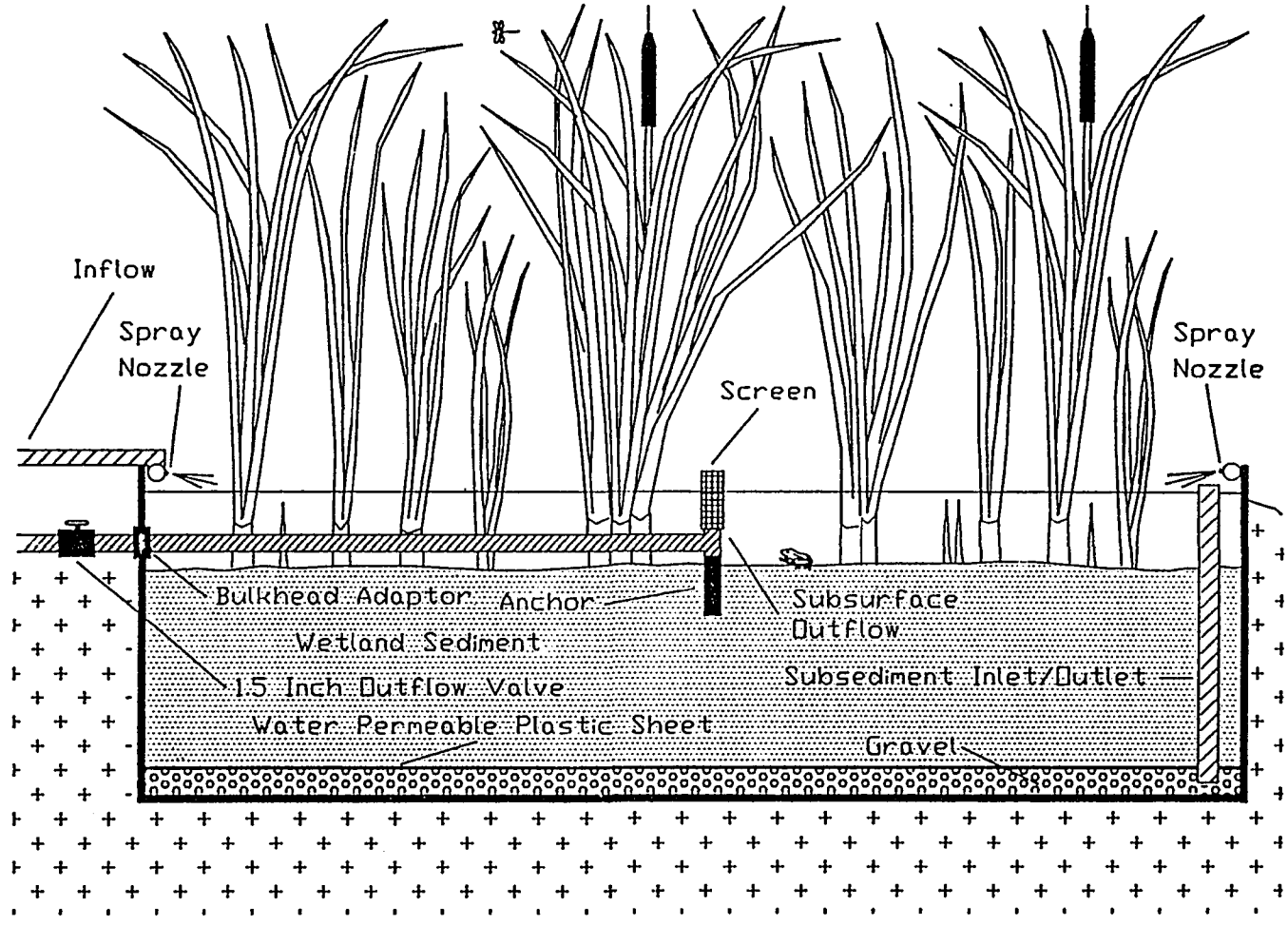

Figure 3. Cross sectional diagram of a mesocosm

Table 1. Chemical characteristics of feedwater to mesocosms.

Calcium (Ca^{+})	100	mg L^{-1}
Magnesium (Mg^{+})	34.2	mg L^{-1}
Sodium (Na^{+})	12.8	mg L^{-1}
Potassium (K^{+})	5.4	mg L^{-1}
Chloride (Cl^{-})	26.7	mg L^{-1}
Sulfate (SO_4^{-})	75.3	mg L^{-1}
Carbonate (CO_3^{2-})	n.d.	mg L^{-1}
Bicarbonate (HCO_3^{-})	357	mg L^{-1}
Total phosphorus	0.059	mg L^{-1} as P
Ortho-phosphorus	0.007	mg L^{-1} as P
Total Kjeldahl nitrogen	0.58	mg L^{-1} as N
Nitrate+nitrite ($NO_3^{-}+NO_2^{-}$)	0.07	mg L^{-1} as N

Whole mesocosm studies

In 1990 and 1991, a series of nitrate batch dose experiments were conducted within the wetland mesocosms to investigate the transformation and fate of externally loaded nitrate within northern prairie wetlands. In all experiments, mesocosms were dosed with sodium nitrate solutions and the decline in nitrate-nitrogen concentration in the overlying water was measured over time. The mesocosms were drained to 5 cm of overlying water and reflooded to a depth of approximately 25 cm with nitrate enriched feedwater. Each mesocosm was sampled by collecting 50 ml of overlying water from three separate locations. These aliquots were composited and a 20 ml subsample was filtered through a 0.2 micron filter and preserved with 0.06 ml concentrated HCl or 0.02 ml concentrated H_2SO_4. Sampling continued until the nitrate-nitrogen concentration in the overlying water dropped below detection limits of about 0.05 mg N L^{-1}. Nitrate-nitrogen was assayed using a second-derivative spectrophotometric procedure (Crumpton et al. 1992). The water level in each mesocosm was recorded daily at the time of sampling to correct for the effect of evapotranspiration on concentration. Rainfall was also recorded daily during sampling periods and assayed for nitrate-nitrogen. All concentrations of nitrate-nitrogen within the mesocosms were corrected for changes related to rainwater dilution or nitrate-nitrogen addition.

The first experimental nitrate addition to thirty six of the mesocosms was performed on August 7, 1990. After addition, the nitrate-nitrogen concentration in the overlying water was 7.3 mg L^{-1}. Each mesocosm was sampled every six hours for the first 48 hours and then every 24 hours thereafter. The second nitrate addition to the same thirty six mesocosms was performed on August 29, 1990. After addition the nitrate-nitrogen concentration in the overlying water was 7.7 mg L^{-1} and each mesocosm was sampled every 24 hours. The third experimental addition of nitrate was made to a subset of eighteen of the mesocosms on October 16, 1990 and samples were again collected from each mesocosm every 24 hours. During a subset of the dosing studies in 1990 one of the mesocosms was instrumented with a water quality monitoring device capable of continuously recording temperature, pH and dissolved oxygen.

Based on initial results from the 1990 season, an effort was made to further define the effects of nitrate loading rate and pattern on the transformation and fate of nitrate. In 1991, twelve mesocosms were assigned to treatments which varied the frequency of nitrate

loading to the systems. Within these mesocosms, four received no nitrate addition at any time throughout the summer, four received additions of NO_3^--N of approximately 10 mg L^{-1} on six occasions throughout the summer and fall during the batch dose experiments (intermediate loading), and four received additions of nitrate frequently enough to continuously maintain an elevated concentration of nitrate (chronic loading). For the chronic loading treatment, a concentrated solution of $NaNO_3$ was applied to each mesocosm with a hand sprayer approximately every third day. The mass of nitrogen applied was equivalent to 10 mg L^{-1} NO_3^--N and the concentration of NO_3^--N in these mesocosms was maintained above 1 mg L^{-1} NO_3^--N throughout the season.

Nitrate addition experiments (intermediate loading) were performed six times during the summer and fall of 1991 (June 12, July 8, August 5, August 28, September 6, and October 30). This allowed an evaluation of seasonal pattern in nitrate transformation rates as well as the comparison of rates with those estimated during 1990. Sampling and analysis procedures used for batch dose experiments were similar to those described for 1990 with a regular sampling interval of 24 hours. During the August 5 batch dose experiment, mesocosms were sampled at sunrise and sunset in addition to the regular sampling interval. During the October 30 batch dose experiment temperatures dropped well below freezing and as much as 15 cm of ice formed on the water surface. Sampling was maintained by drilling three holes through the ice and compositing these samples.

Vegetation and ^{15}N studies

In conjunction with the September 6, 1991 dosing experiment, stable nitrogen isotope (^{15}N) tracer experiments were conducted within enclosures inserted to isolate portions of the experimental wetland mesocosms. The enclosures are designed for short term experiments and allow many more manipulations than are possible in the whole mesocosms. The enclosures consist of 75 cm diameter polyethylene cylinders, 90 cm tall, with a wall thickness of 0.3 cm. The enclosures were driven at least six cm into the sediment of the mesocosm, enclosing 0.44 m^2 of intact sediment and approximately 112 L of overlying water.

Enclosures were inserted into each of three of the mesocosms receiving intermediate and chronic nitrate loading patterns. As part of the routine dosing experiment conducted on September 5, 1991, the mesocosms were drained to 5 cm of overlying water and

reflooded to a depth of approximately 25 cm with nitrate enriched feedwater. The concentration of NO_3^--N in the mesocosms after addition was approximately 9 mg L^{-1}. Simultaneously, the water within the polyethylene enclosures was pumped out and replaced with $^{15}NO_3^-$ and Cl enriched water. The $K^{15}NO_3^-$ enriched water (32 atom % ^{15}N) was made in batch to contain approximately 9 mg L^{-1} $^{15}NO_3^-$-N and 85 mg L^{-1} NaCl-Cl. The chloride was added as a conservative tracer to estimate if there was any transfer of solution between the water within the enclosure and the whole mesocosm or if simple diffusion into the sediment was significant. The chloride concentration added within the enclosures was approximately twice the concentration of that in the whole mesocosm.

The enclosures were sampled by collecting 50 ml of overlying water from three separate locations. These aliquots were composited and a 20 ml subsample was filtered through a 0.2 micron filter and preserved with 0.02 ml concentrated H_2SO_4. Each mesocosm and enclosure was sampled daily until the nitrate concentration in the overlying water dropped below detection limits of about 0.05 mg N L^{-1}. NO_3^--N was assayed using a second-derivative spectrophotometric procedure (Crumpton et al. 1992) and Cl was assayed by titration using the argentometric method (American Public Health Association 1989).

When the concentration of nitrate had dropped below detection limits, the contents of the enclosures were destructively sampled to determine the fate of the added $^{15}NO_3^-$. Live cattails were cut off at the sediment surface and divided into an older, reproductive cohort (live old), and a newer, purely vegetative cohort (live new). Dead standing cattails were cut off at the sediment surface. Cattail litter was separated and collected and cattail roots were cut off at the sediment surface. The water was then pumped out of enclosures at the same time that the mesocosms were drained. The top two cm of sediment within the enclosures was removed, homogenized, and subsampled. Cattail rhizomes were removed from within the area of the enclosures and the remaining sediment below two cm homogenized and subsampled. Total cattail densities within each mesocosm were determined in November 1991.

Sediment and water samples for ^{15}N analyses were frozen immediately upon return to the laboratory. The sediment was then lyophilized, subsampled, and finely ground using a mortar and pestle. Rhizomes, litter and root samples were gently washed to remove adhering sediment and oven-dried at 80°C to constant weight. After weighing, the plant

samples were coarse ground in a 10-mesh Wiley mill, subsampled, and finely ground in a 60-mesh Wiley mill. Carbon and nitrogen contents of plant fractions were assayed using a Carlo Erba NA1500 N/C/S analyzer.

Determination of ^{15}N percentages was carried out in the laboratory of Dr. Alfred Blackmer of the Department of Agronomy at Iowa State University. Procedures are as described by Sanchez and Blackmer (1988). Exchangeable ammonium-N and nitrate (plus nitrite)-N contents of each sediment sample were determined by extraction with 2 N KCl and steam distillation with magnesium oxide and Devarda alloy as described by Keeney and Nelson (1982). Because distillate from these analyses were used for ^{15}N determinations, 5 ml of an ammonium nitrate standard containing 15 ug ammonium-N ml^{-1} was added to each aliquot (20 ml) of sediment extract distilled. This practice assured that each sample contained enough N to be within the working range of the mass spectrometer used for ^{15}N determinations. Distillates from the first aliquots were collected in boric acid indicator solution and then titrated with acid as described by Keeney and Nelson (1982). Distillates from the second aliquots were collected in 2 ml of 0.08 N H_2SO_4, concentrated by evaporation of water to a volume of about 2 ml, and stored for analysis of ^{15}N. The permanganate-reduced iron modification of the Kjeldahl procedure (Bremner and Mulvaney 1982) was used to determine total nitrogen contents of sediment and plant tissue samples.

Determinations of ^{15}N in sediments, sediment extracts, and plant samples were performed by reacting the concentrated distillates with sodium hypobromite in evacuated Rittenburg flasks as described by Hauck (1982) and injecting the resulting dinitrogen gas into a Varian MAT 250 mass spectrometer. Atom percentages ^{15}N in these distillates, concentrations of ^{15}N-derived nitrate and ammonium nitrogen, and concentrations of ^{15}N-derived total nitrogen were calculated as in Sanchez and Blackmer (1988).

Statistical calculations follow Steel and Torrie (1980) and Day and Quinn (1989). Analysis of variance and orthogonal planned comparisons were used to determine significance of treatments. Differences between means for ANOVA and planned comparisons were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Whole mesocosm studies

All of the mesocosm studies during 1990 and 1991 confirm the considerable capacity of wetlands to remove nitrate. Even under highly aerobic conditions, nitrate concentrations declined rapidly in all of the mesocosm experiments (Figures 4 and 5). When dosed with approximately 10 mg L^{-1} NO_3^--N, (the MCL for drinking water), the experimental wetlands generally reduced the nitrate concentration to near detection limits within five days. The only exceptions to this were dosing experiments conducted late in the season when temperatures were much lower.

However, as the dosing experiment conducted on October 30, 1991 demonstrates, appreciable nitrate loss occurs within these systems even when water temperatures are near freezing. During this experiment the initial nitrate concentration within all treatment mesocosms was 13.7 mg NO_3^--N L^{-1}. On the second day after dosing, nearly 7.6 cm of rain caused a rapid drop in nitrate concentration, from an average of 12.6 mg NO_3^--N L^{-1} on day 1 to 9.6 mg NO_3^--N L^{-1} on day 2. On the third day after dosing, temperatures plummeted to well below freezing and ice rapidly developed. Nitrate concentration increased over the next two days up to 11.5 mg NO_3^--N L^{-1}. The ice reached its greatest thickness of nearly 15 cm on day 11 and was nearly all melted again by day 18. Between days 4 and 11, when the ice was thickening, the concentration of nitrate slowly declined from an average within treatment mesocosms of 11.5 mg NO_3^--N L^{-1} to 9.0 mg NO_3^--N L^{-1}. After day 11, as the ice was decreasing in thickness, the concentration of nitrate declined more rapidly and was approaching detection limits by day 23.

Rates of nitrate loss on a sediment area basis (g N m^{-2} day^{-1}) observed within these experimental wetland mesocosms are among the highest reported in the literature for any wetland system (Seitzinger 1990, Johnston 1991). Nitrate loss rates recorded during 1990 ranged around 0.5 to 0.8 grams of nitrate-nitrogen per square meter of sediment per day in the presence of several mg nitrate-N L^{-1} or more. During 1991, nitrate loss rates were consistently higher, reaching over 1.5 g N m^{-2} day^{-1} during the July 8 dosing. This may reflect the maturation of the mesocosms as ecosystems, in particular the buildup of decaying cattail litter which provides anaerobic microsites necessary for nitrate reduction (Isenhart 1992).

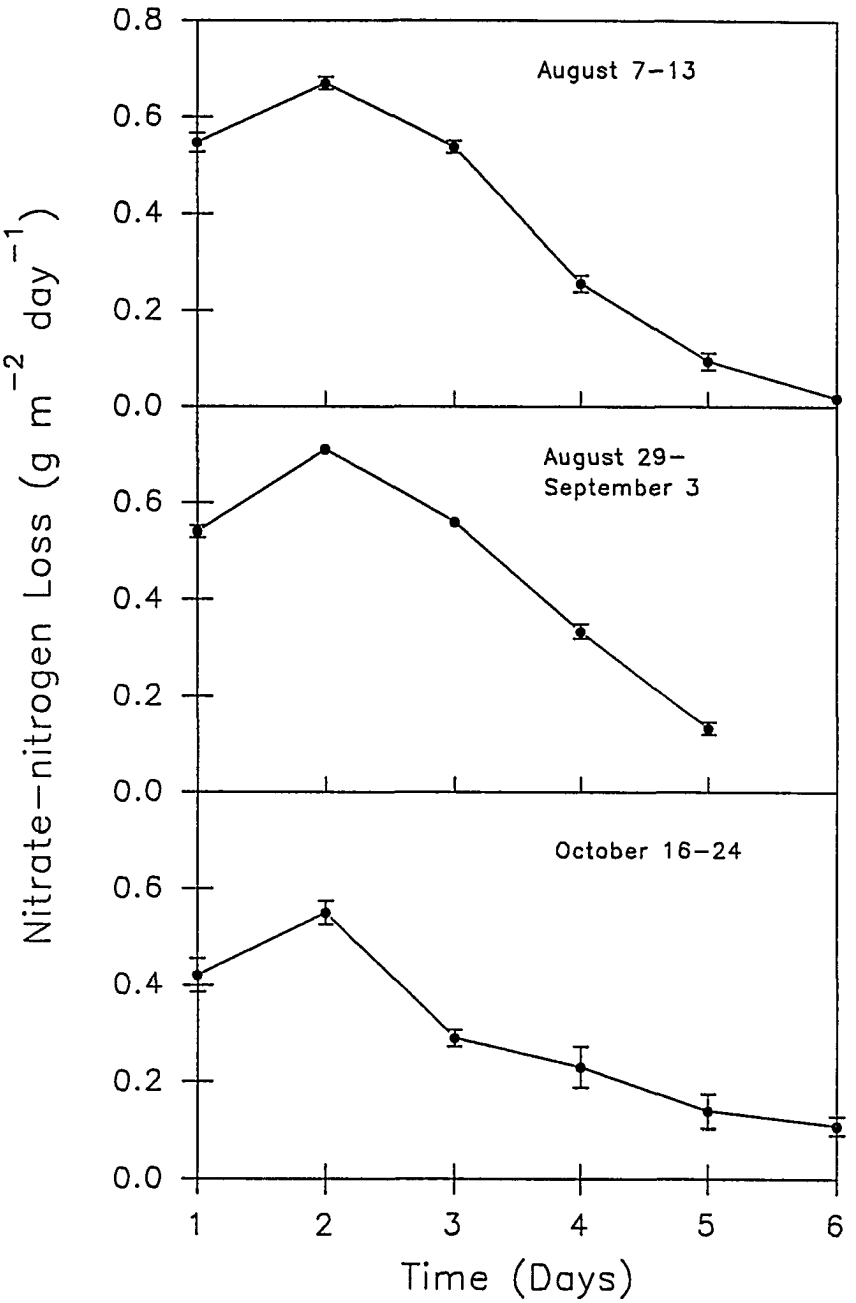

Figure 4. Nitrate-nitrogen concentration in mesocosms following experimental additions in 1990. Error bars indicate $\pm$ one standard error (n = 36 on Aug. 7 and Aug. 29, n = 18 on Oct 29)

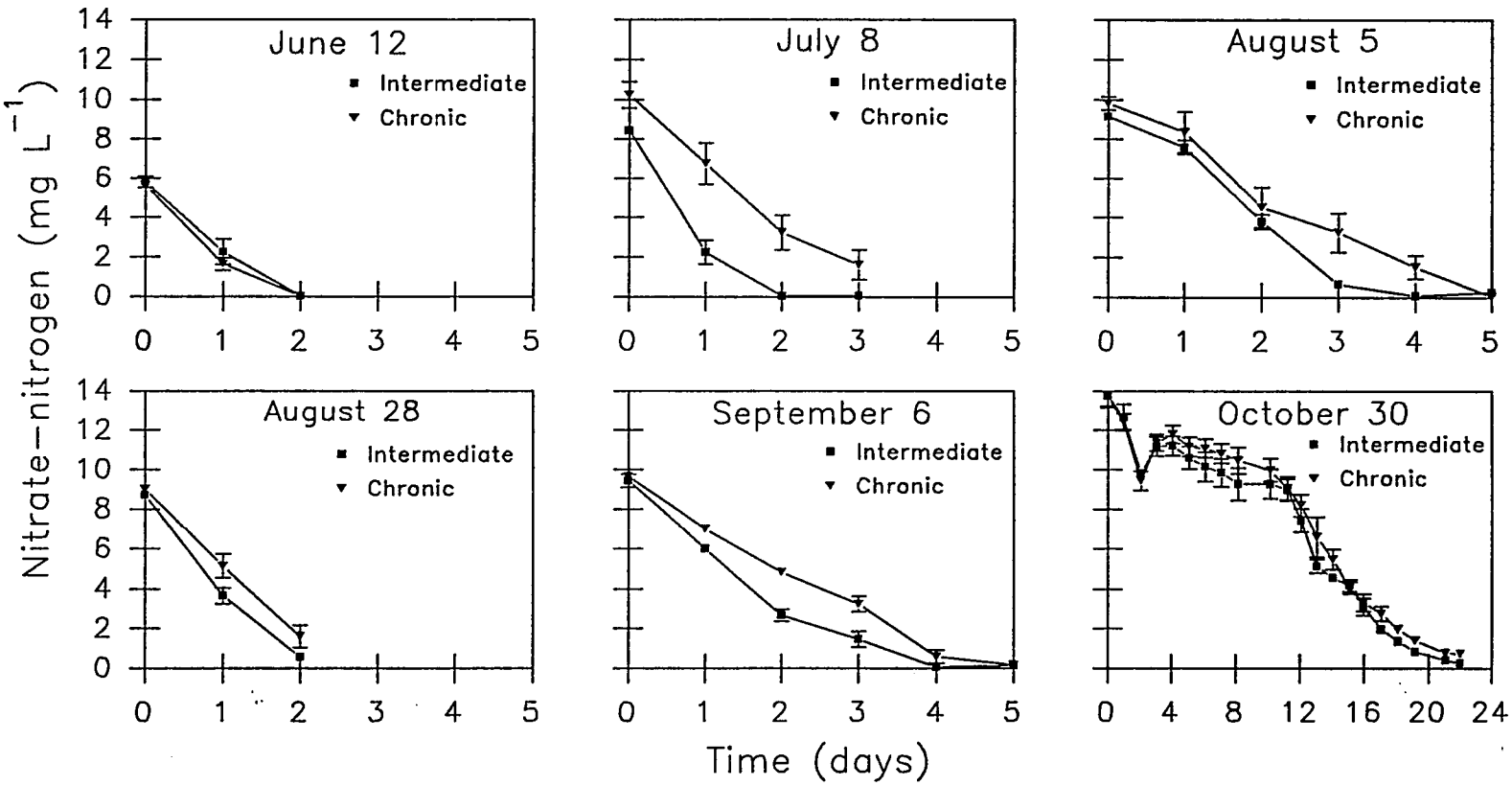

Figure 5. Nitrate-nitrogen concentration in mesocosms following experimental additions in 1991. Error bars indicate $\pm$ one standard error (n = 4)

Nitrate loss rates in all mesocosm experiments in 1990 reached a peak in the range of 0.6 to 0.7 g N m^{-2} day^{-2} on the second day after addition (Figure 6). This indicates that there was a period of equilibration required following the replacement of the water in the mesocosms during experimental addition. The nitrate enriched feedwater added to mesocosms was generally colder than the water it replaced and approximately 24 hours was required for temperature equilibration (Figure 7). Nitrate loss rates decline in the following days, coincident with the decrease in concentration of nitrate in the overlying water. This pattern was quite clear for the first two mesocosm experiments but less so for the third experiment during which nitrate concentrations declined more slowly, perhaps related to the lower temperatures during the third experiment. In contrast to 1990, with the exception of the August 5 experiment, the nitrate loss rate in all experiments in 1991 was highest on the first day after addition (Figure 8). Nitrate loss rate then declines in the following days, again coincident with the decrease in nitrate concentration in the overlying water.

The effect of nitrate concentration within the overlying water on nitrate loss rate is illustrated in Figure 9 which includes data from all mesocosms during the August 29, 1990 dosing experiment. Nitrate loss rates are clearly a function of the concentration of nitrate in the overlying water over a wide range of concentrations. This is consistent with models of denitrification in agricultural streams which suggest that in the presence of high external nitrate loads, denitrification rates are limited by substrate transport and controlled by the nitrate concentration in the overlying water and the effective length of the diffusion path between the overlying water and anaerobic sites of denitrification (Christensen et al. 1990). Higher concentrations of nitrate in the overlying water of the mesocosms increases the rate of nitrate diffusion to anaerobic sites, resulting in higher nitrate loss rates. Nitrate loss rates then decline coincident with the concentration of nitrate, reflecting the lower diffusion gradient.

Factors controlling the effective length of the diffusion path of nitrate to anaerobic sites include temperature, the effective surface area of sediment and litter to provide anaerobic sites, and oxygen concentrations. Within the mesocosms, temperature can be expected to demonstrate daily as well as seasonal fluctuations (Figure 7). The effect of temperature is illustrated by the lower nitrate loss rates during the mesocosm dosing experiment conducted in October 1990. It is unclear from these studies, however, whether

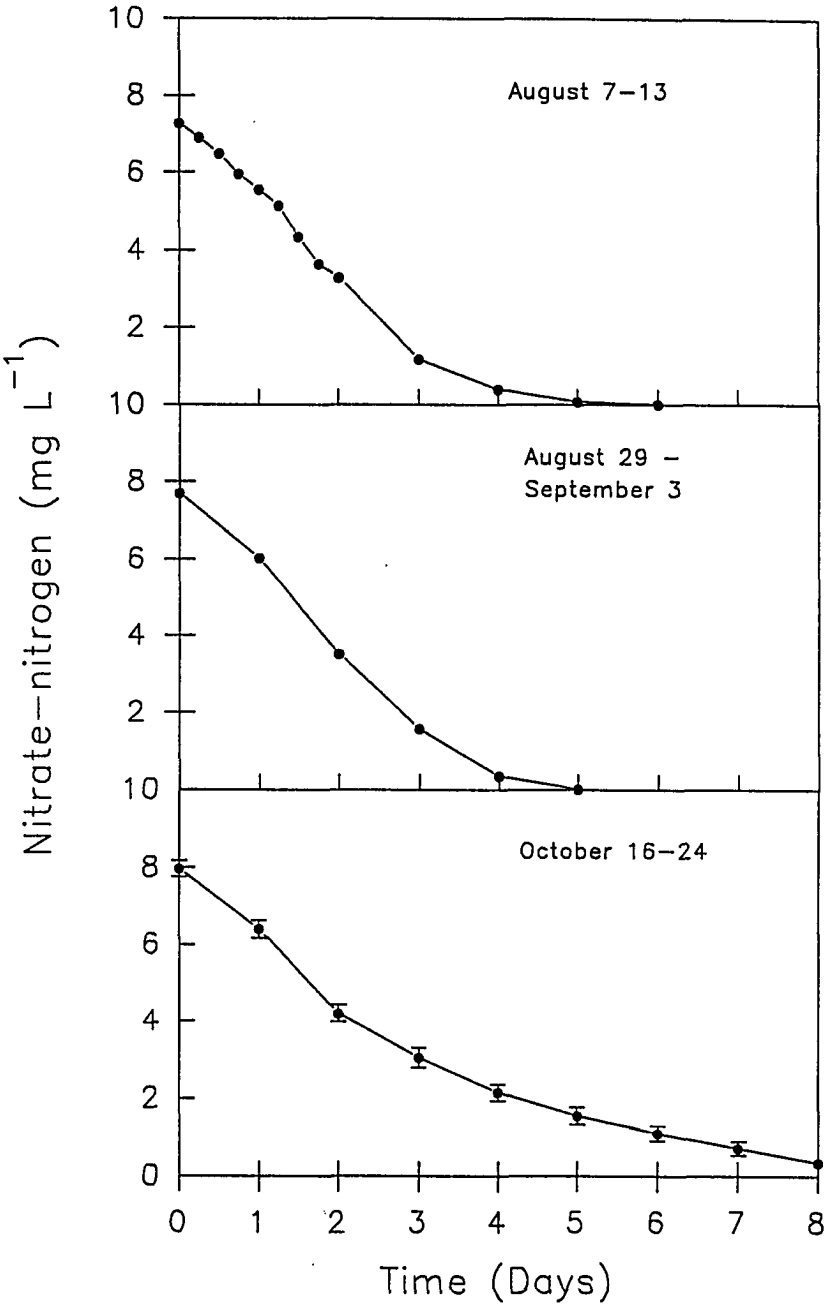

Figure 6. Nitrate-nitrogen loss rate in mesocosms following experimental additions in 1990. Error bars indicate $\pm$ one standard error (n = 36 on Aug. 7 and Aug 29, n = 18 on Oct. 29)

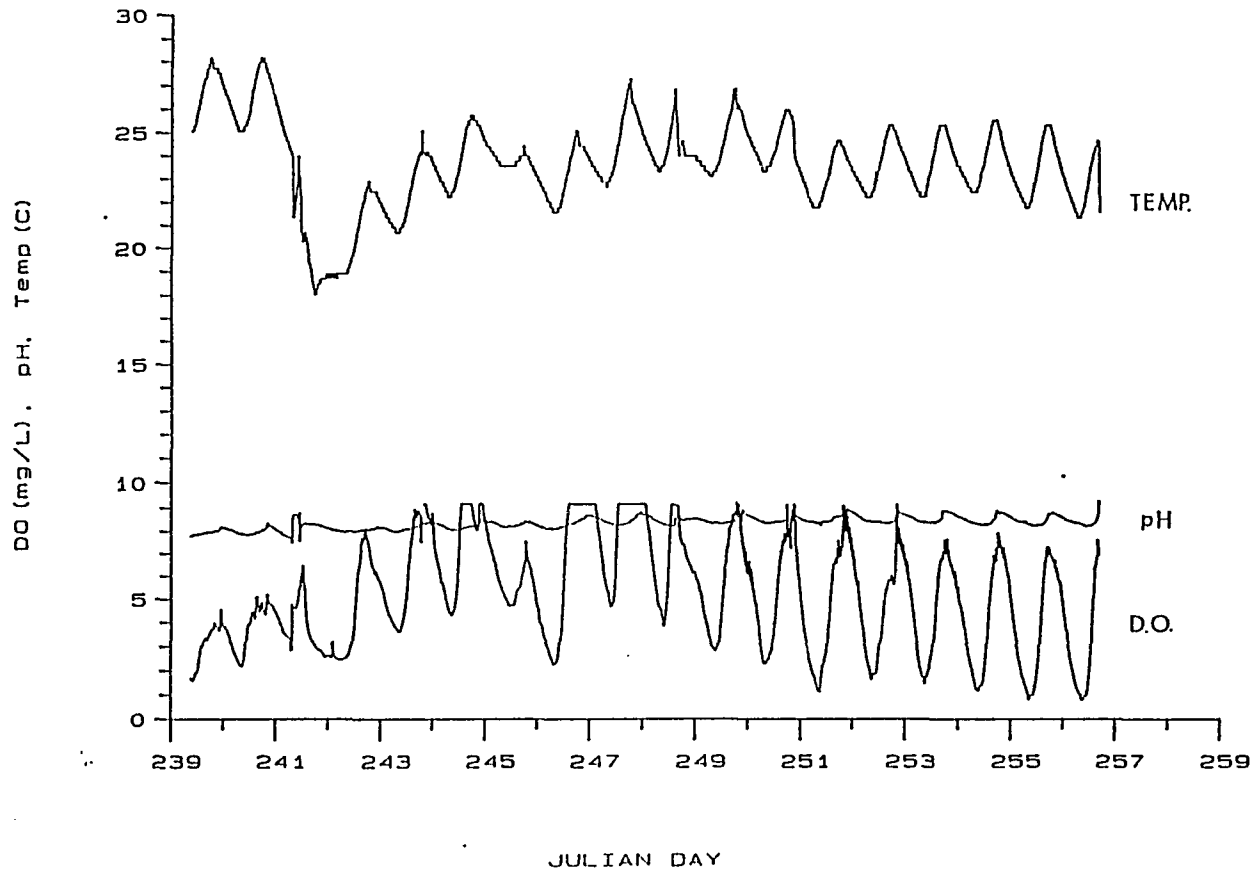

Figure 7. Dissolved oxygen (mg L^{-1}), pH, and temperature (^{o}C) in a representative mesocosm during the August 29, 1990 mesocosm batch dose study. Experimental nitrate addition was made on Julian day 241

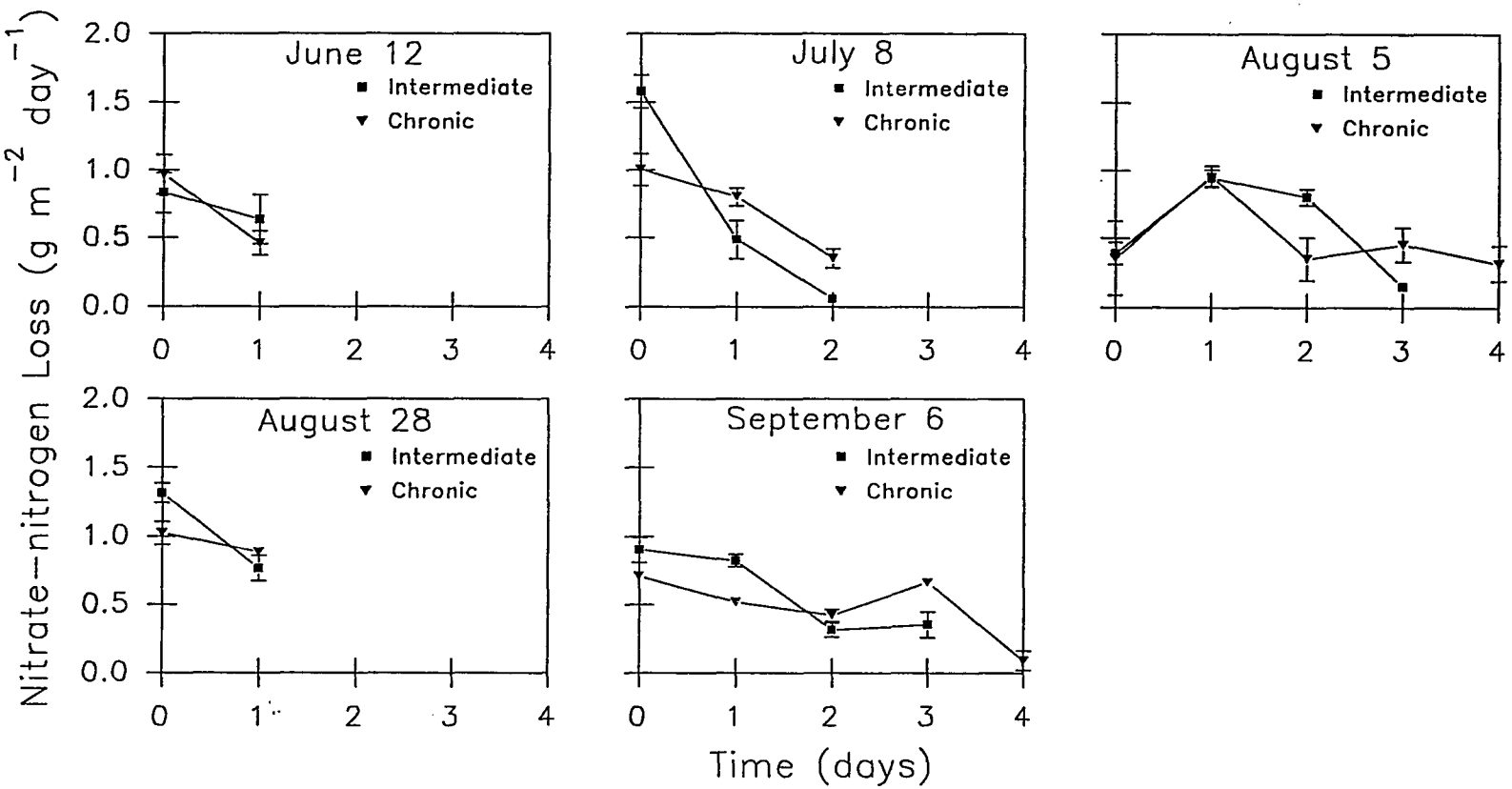

Figure 8. Nitrate-nitrogen loss rate in mesocosms following experimental additions in 1991. Error bars indicate ± one standard error (n = 4)

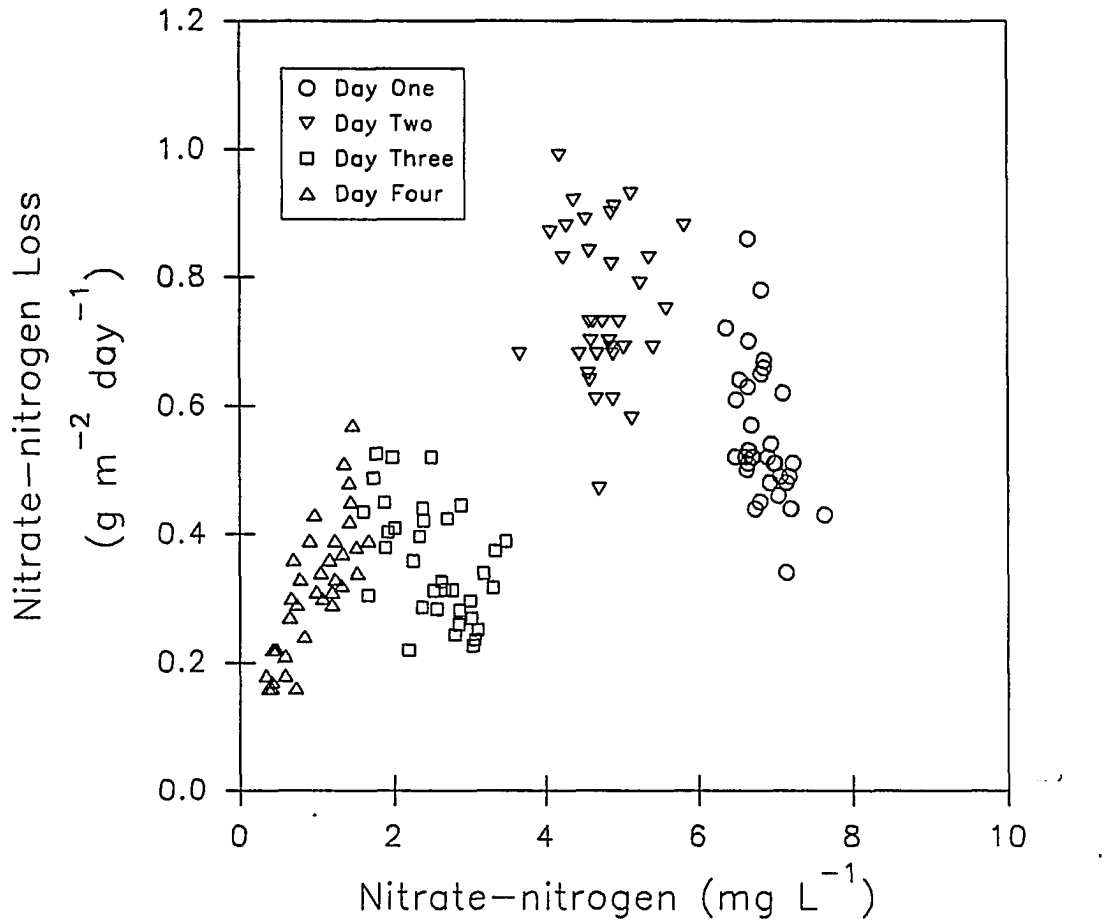

Figure 9. Nitrate-nitrogen loss rate versus nitrate-nitrogen concentration for the first four days after experimental nitrate addition on August 29, 1990

temperature effects are manifested predominantly in rates of nitrate and oxygen diffusion or in rates of metabolic processes.

Oxygen concentration has also been demonstrated to be an important factor limiting nitrate loss via denitrification under certain conditions (Tiedje 1989). The oxygen status of a habitat is controlled by the rate of oxygen supply to that site through diffusion or production by photosynthesis and the rate of oxygen consumption by respiration. Within the mesocosms, dissolved oxygen demonstrates dramatic daily as well as seasonal fluctuations (Figure 7). The dissolved oxygen concentration is shown to vary by as much 8 mg L^{-1} within a 24 hour period. Such shifts in dissolved oxygen, can be expected to have significant effects on nitrate loss rates.

Results of the sunrise-sunset samplings taken during the August 5, 1991 dosing experiment illustrate the control that light exposure has on the nitrate loss rate within the mesocosms (Figure 10). Rates of nitrate disappearance (g N m^{-2} day^{-1}) within the mesocosms demonstrate a distinct diurnal pattern. During the day, when photosynthesis elevates the concentration of dissolved oxygen within the mesocosms, the nitrate loss rate is lower. At night, when dissolved oxygen concentrations decline, the nitrate loss rate is higher. The overall negative slope apparent in nitrate loss rate is again the result of the declining nitrate concentration in the overlying water.

Such diurnal patterns in nitrate loss rate can be explained by algal photosynthetic oxygen production causing a deeper extension of oxic surface zones in biofilms and sediments (Sorensen and Revsbech 1990). This increases the diffusion path of externally loaded nitrate to underlying anaerobic zones which are the primary sites of denitrification, resulting in lower nitrate loss rates. The absence of photosynthetic oxygen production will thus result in higher nitrate loss rates during the night. While previous researchers have also documented lower denitrification rates in aquatic systems during the day than at night (Andersen et al. 1984, Nielsen et al. 1990 a,b), these studies have generally been conducted in the laboratory in sediment-water microcosms. The studies conducted within the wetland mesocosms provide realistic, ecosystem level estimates of the effects of photosynthetic oxygen production on denitrification rates.

Rates of nitrate removal per unit area of wetland are dependent upon both the concentration of nitrate in the overlying water and the specific rate or nitrate removal

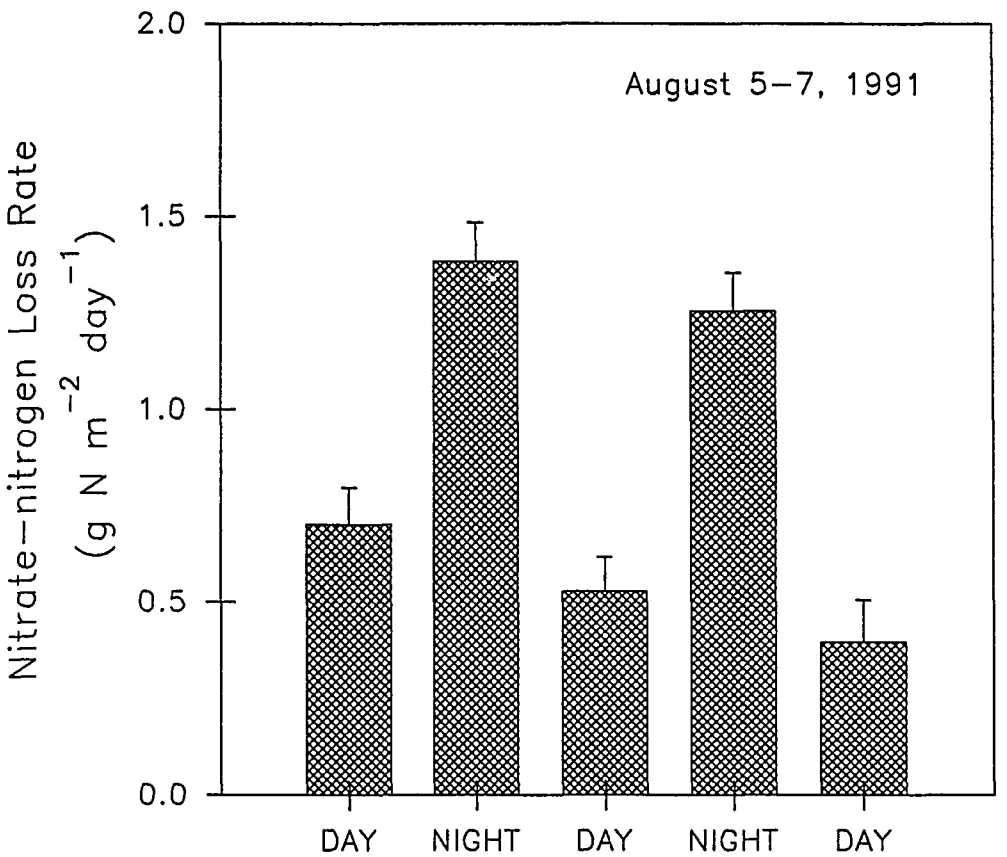

Figure 10. Diurnal pattern in nitrate-nitrogen loss in mesocosms as measured from sunrise and sunset samplings during 1991. Error bars indicate $\pm$ one standard error (n = 8)

capacity of the wetland. These parameters can be related by the equation F = vg * C where F is the nitrate loss rate and C is the average nitrate concentration in the overlying water. The velocity of deposition (vg) of nitrogen for each mesocosm for each experiment was calculated based on the following formula:

$$vg = F/C$$

Where

F= the loss rate of nitrate for each period in g N m^{-2} day^{-1} and,

C= the average concentration of nitrate in g N m^{-3}

Vg, then, is the velocity of deposition in m day^{-1} and expresses the nitrate removal capacity of the wetland.

Velocities of deposition for each mesocosm experiment during 1990 are shown in Figure 11. The velocity of deposition generally increased over time since addition during the August 7 and August 23 experiments. During the October 16 experiment, the velocity of deposition was lower and did not demonstrate the same pattern of increase over time, likely a result of temperatures being much lower during the third experiment than in the first two experiments.

Mesocosm experiments conducted during 1990 demonstrated that the velocity of deposition increased during each of the first two mesocosm experiments, coincident with the decline in nitrate concentrations. The increase in the velocity of deposition during each of these experiments suggested that nitrate addition stimulated increases in activities and/or population densities of denitrifying bacteria. This was also consistent with the lower and more stable velocity of deposition observed during the last mesocosm experiment, since the lower temperatures during that experiment would have slowed population growth. These patterns suggest that the nitrate removal capacity of wetlands might be greatly enhanced if nitrate loads are sufficient to maintain high population densities of denitrifying organisms.

The nitrate loading treatments imparted on a subset of the mesocosms during 1991 were intended to further investigate this relationship between nitrate loading pattern and nitrate loss in wetland systems. In those mesocosms subjected to only occasional

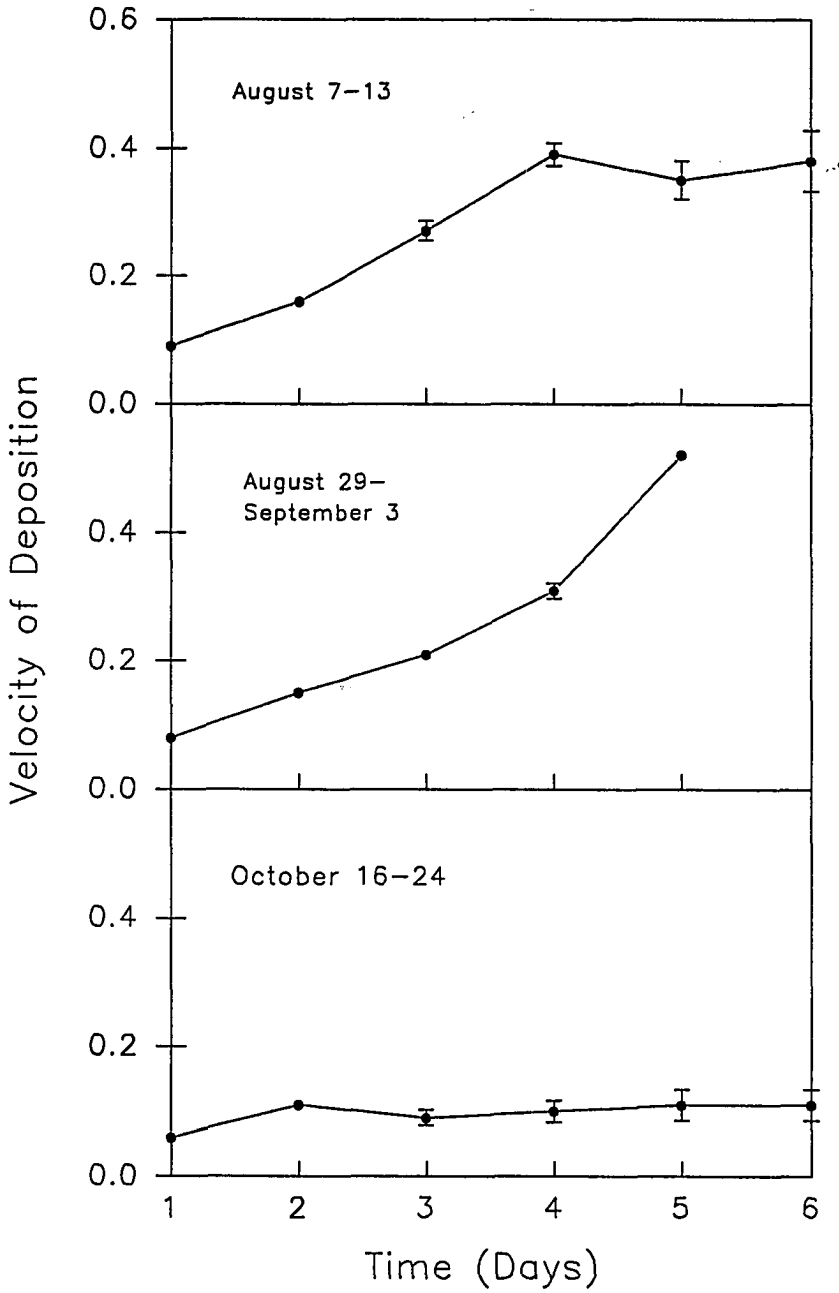

Figure 11. Velocity of deposition (vg) of nitrate-nitrogen in mesocosms following experimental addition in 1990. Error bars indicate $\pm$ one standard error (n = 36 on Aug. 7 and Aug. 29, n = 18 on Oct 29)

(intermediate) nitrate loadings, it was expected that the pattern in velocity of deposition would be similar to that observed in 1990. In those mesocosms in which the concentration of nitrate was kept consistently elevated (chronic), it was expected that the observed velocity of deposition would initially be higher than in less exposed systems and would demonstrate no pattern during the dosing experiments.

Patterns in nitrate loss and velocity of deposition observed during 1991, however, did not confirm these expectations. When comparing patterns in nitrate decline (Figure 5), there was no difference in nitrate loss between treated mesocosms during the June 12 dosing, which marked the initiation of loading treatments. After the initiation of the nitrate loading treatments, however, the concentration of nitrate within the mesocosms declined more rapidly in those mesocosms which were subjected to the intermediate nitrate loading pattern versus the chronic pattern during each of the July 8, August 5, August 28, and September 6 dosing experiments. Nitrate loss rates were thus initially higher in those mesocosms subjected to the intermediate nitrate loading rate. As the concentration of nitrate was depleted faster in the intermediate loading mesocosms, however, nitrate loss rates remained higher in those mesocosms subjected to the chronic nitrate loading. There was no discernible pattern in velocity of deposition estimates for the mesocosm batch dose experiments conducted during 1991 (Figure 12).

These results indicate that while nitrate exposure may indeed stimulate increases in activities and/or population densities of denitrifying bacteria within wetland systems that have received little or no prior nitrate exposure, other factors quickly become more proximate in the control of nitrate loss. For example, the faster nitrate removal observed during 1991 in those mesocosms exposed to the intermediate nitrate loading might be explained by a greater unmet assimilatory nitrogen demand within these systems. In those mesocosms exposed to chronic nitrate loading, this assimilatory nitrogen demand may be suppressed, resulting in slower nitrate removal.

Vegetation studies

The effect of nitrate loading on vegetation quantity and quality was examined by comparing treated mesocosms against control mesocosms which had also been drained and subsequently refilled with unamended feedwater on each of the dates of the batch dose experiments. If the overall effect of nitrate loading was significant, a planned comparison

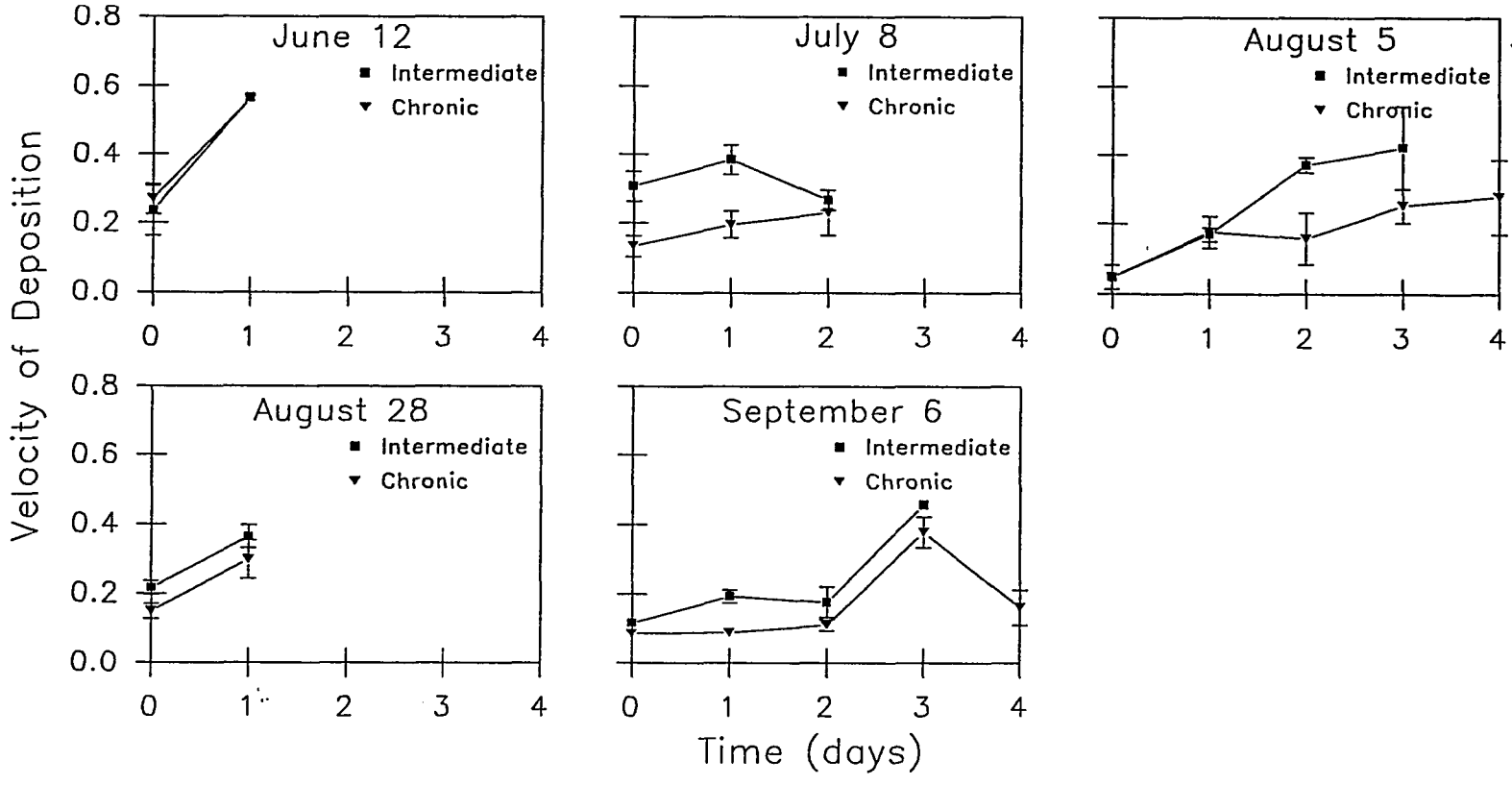

Figure 12. Velocity of deposition (vg) of nitrate-nitrogen in mesocosms following experimental addition in 1991. Error bars indicate $\pm$ one standard error (n = 4)

was used to determine if there was a significant difference between the intermediate and chronic nitrate loading rates.

Nitrate loading induced a significant response in the quantity of *Typha* within the mesocosms but the pattern is difficult to interpret (Figure 13). The lower density of cattails within intermediate loading mesocosms compared to either zero or chronically loaded mesocosms was significant, yet there was no significant difference in cattail densities between control and chronically loaded mesocosms.

In those mesocosms that received chronic nitrate loading, an additional, purely vegetative cohort of cattails grew late in the season (identified as live new cattails in Figure 14). This effect of overall nitrate loading was significant, as was the effect of nitrate loading rate, with those mesocosms receiving the chronic nitrate loading having a significantly greater biomass of live new cattails. At the time of the $^{15}NO_3^-$ experiment, the earlier cohort of cattails had begun to senesce while this later cohort remained a lush green. This suggests that *Typha* is capable of utilizing the externally loaded nitrate as a nitrogen source in meeting assimilatory demands. There was no significant difference in response to nitrate loading in the mass dry weight per area of any of the other plant fractions.

Addition of nitrate to the mesocosms also had a significant effect on carbon and nitrogen content and carbon/nitrogen ratio of several plant fractions (Table 2). The difference in percent nitrogen in treated versus control mesocosms was significant in the live old cattail and rhizomes fractions, with percent nitrogen also significantly greater in those mesocosms subjected to the chronic nitrate loading rate. The difference in percent carbon in treated versus control mesocosms was significant only in the dead floating and rhizome fractions. There was, however, no significant difference in percent carbon between the intermediate and chronic nitrate loading rates in these two fractions.

The difference in carbon/nitrogen ratio in the live old and rhizome fractions in treated versus control mesocosms was also significant. Within these two fractions, there was also a significant difference in carbon/nitrogen ratio between the loading rates, with live old cattails and rhizomes in those mesocosms receiving the chronic nitrate loading having a lower carbon/nitrogen ratio (Table 2). The greater percent nitrogen and lower carbon/nitrogen ratio of these fractions in the mesocosms subjected to the higher nitrate

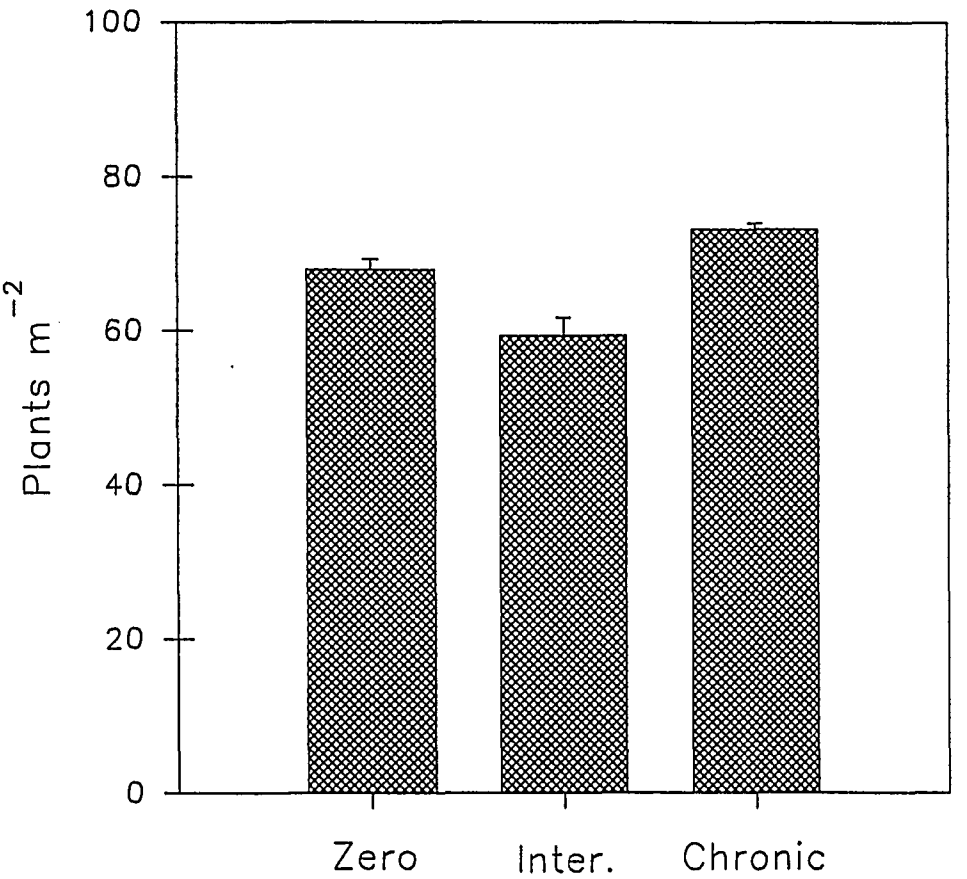

Figure 13. Densities of *Typha glauca* (cattail) within mesocosms receiving zero, intermediate, or chronic nitrate loading. Error bars indicate $\pm$ one standard error (n = 4)

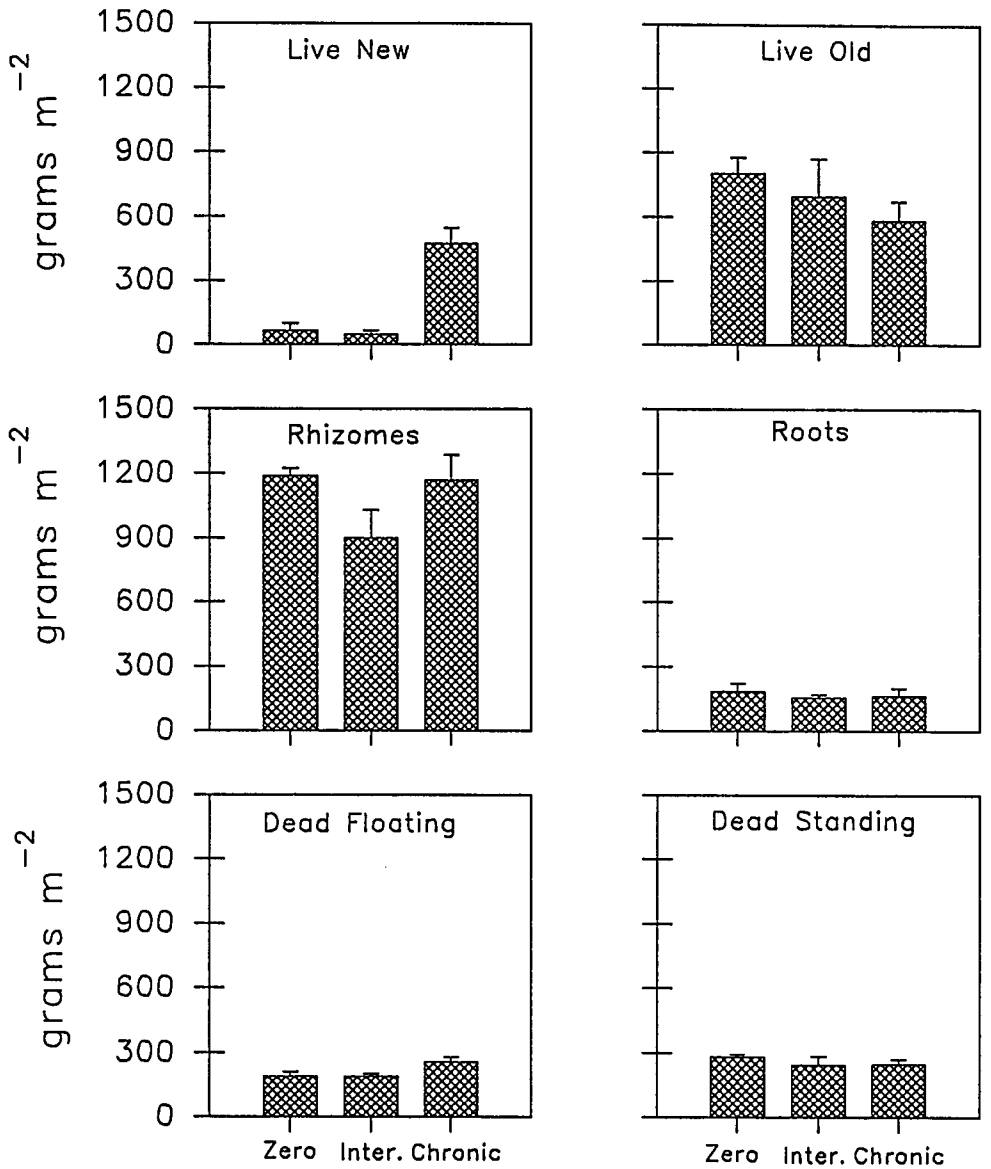

Figure 14. Biomass of *Typha glauca* (cattail) fractions within mesocosms receiving zero, intermediate, or chronic nitrate loading. Error bars indicate $\pm$ one standard error (n = 3)

Table 2. Percent carbon, percent nitrogen, and carbon/nitrogen ratio of *Typha glauca* (cattail) fractions within mesocosms receiving nitrate loading treatments.

Fraction	Loading Pattern	Percent Carbon		Percent Nitrogen		C/N		n
		Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	
Dead Floating	Control	45.36	0.64	1.37	0.11	34.2	3.3	4
	Intermediate	42.24	0.52	1.45	0.04	29.3	1.0	4
	Chronic	43.11	0.79	1.58	0.04	27.4	0.8	4
Dead Standing	Control	47.57	0.25	0.50	0.02	96.6	4.0	4
	Intermediate	46.54	0.51	0.41	0.03	115.9	6.6	4
	Chronic	46.97	0.12	0.56	0.02	83.6	2.9	4
Live New	Control	47.47	0.18	1.26	0.09	38.2	2.6	3
	Intermediate	47.25	0.48	1.54	0.10	31.0	1.8	3
	Chronic	46.45	0.43	1.40	0.09	33.6	2.0	3
Live Old	Control	46.33	0.19	0.63	0.04	74.7	4.6	3
	Intermediate	47.17	0.37	0.75	0.03	63.1	2.2	3
	Chronic	46.47	0.06	0.99	0.03	47.0	1.7	3
Rhizomes	Control	39.86	0.30	0.58	0.04	69.9	4.8	4
	Intermediate	40.55	0.41	0.67	0.05	61.3	3.9	4
	Chronic	39.56	0.96	1.08	0.03	36.5	0.4	4
Roots	Control	35.34	2.50	1.19	0.05	29.6	1.3	4
	Intermediate	33.57	4.10	1.10	0.09	30.1	1.3	4
	Chronic	31.55	2.99	1.08	0.06	29.3	2.3	4

loading rates would also suggest that the plants are taking advantage of the externally loaded nitrate through assimilation and immobilization.

^{15}N studies

Nitrate concentration in the overlying water decreased rapidly after addition in both the mesocosms and the polyethylene enclosures (Figure 15). At the same time, there was no significant difference in chloride concentration in the overlying water within the enclosures over the course of the experiment (Figure 16), indicating that there is no significant exchange of water between the mesocosms and the polyethylene enclosures. Nitrate concentration in the overlying water of the mesocosms did decline faster than within the enclosures in both treatments (Figure 15). This rate effect, however, was likely not great enough to differentially effect the fate of the loaded nitrate inside or outside the enclosures.

The ^{15}N tracer studies were intended to determine the fate of externally loaded nitrate within the wetland mesocosms as well as to estimate the effect of nitrate loading pattern on nitrate fate. Data obtained from the ^{15}N studies were analyzed in two ways. Initially, the atom percent enrichments within the various plant and sediment nitrogen fractions are compared to natural abundance estimates obtained from control mesocosms to determine if there was a significant overall enrichment related to the added $^{15}NO_3^-$. An enrichment in the atom percent ^{15}N within a nitrogen fraction indicates incorporation of nitrogen derived from the added $^{15}NO_3^-$. If the overall effect of nitrate loading was significant, a planned comparison was used to determine the significance of differences related to nitrate loading rate.

Secondly, the atom percent enrichments were combined with the weights of each plant or sediment fraction and used to calculate a mass balance of the amount of ^{15}N remaining in each fraction within the enclosures compared to the amount of ^{15}N added as $^{15}NO_3^-$. Differences in mass of $^{15}NO_3^-$ incorporated within a fraction will thus be the result of either a higher ^{15}N enrichment combined with equal fraction weights, or the same ^{15}N enrichment combined with unequal fraction weights.

Patterns observed in the percent ^{15}N enrichment of the various plant fractions (Table 3) indicate a larger unmet assimilatory nitrogen demand in those systems receiving

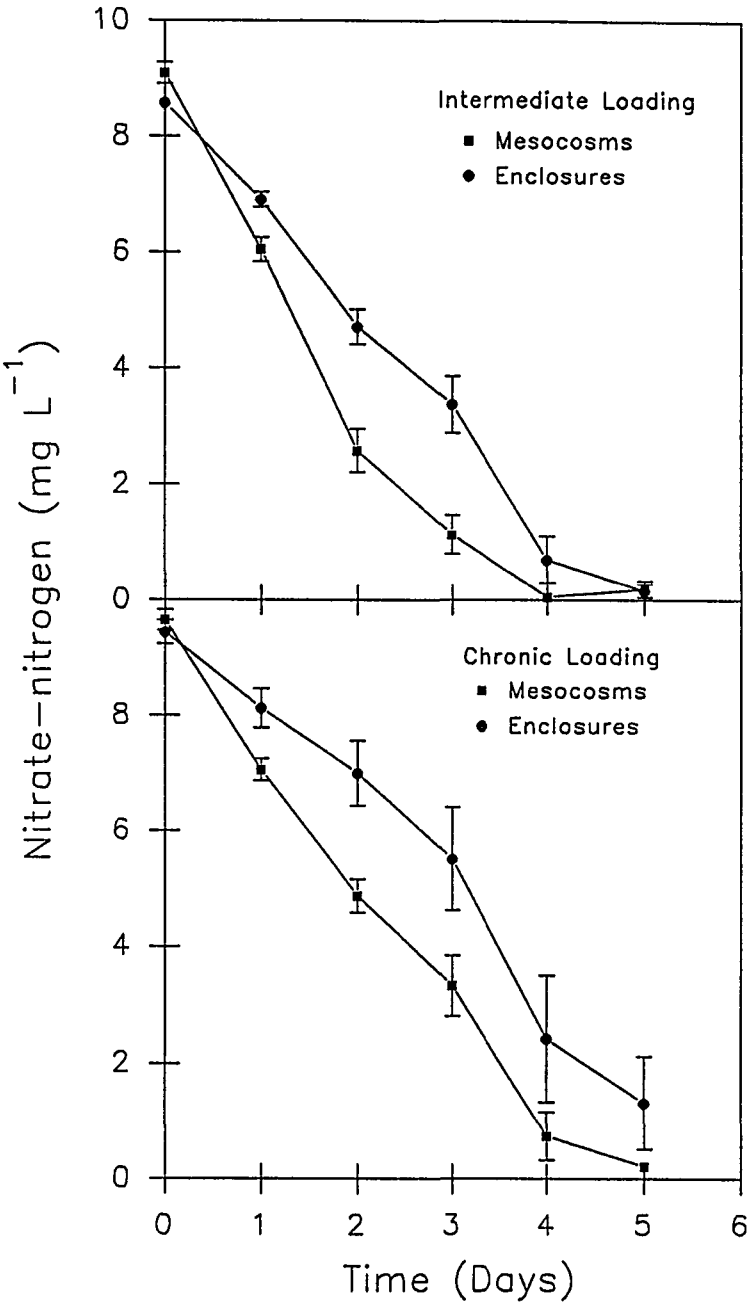

Figure 15. Nitrate-nitrogen concentration in mesocosms and experimental enclosures following experimental nitrate addition. Error bars indicate $\pm$ one standard error (n = 3)

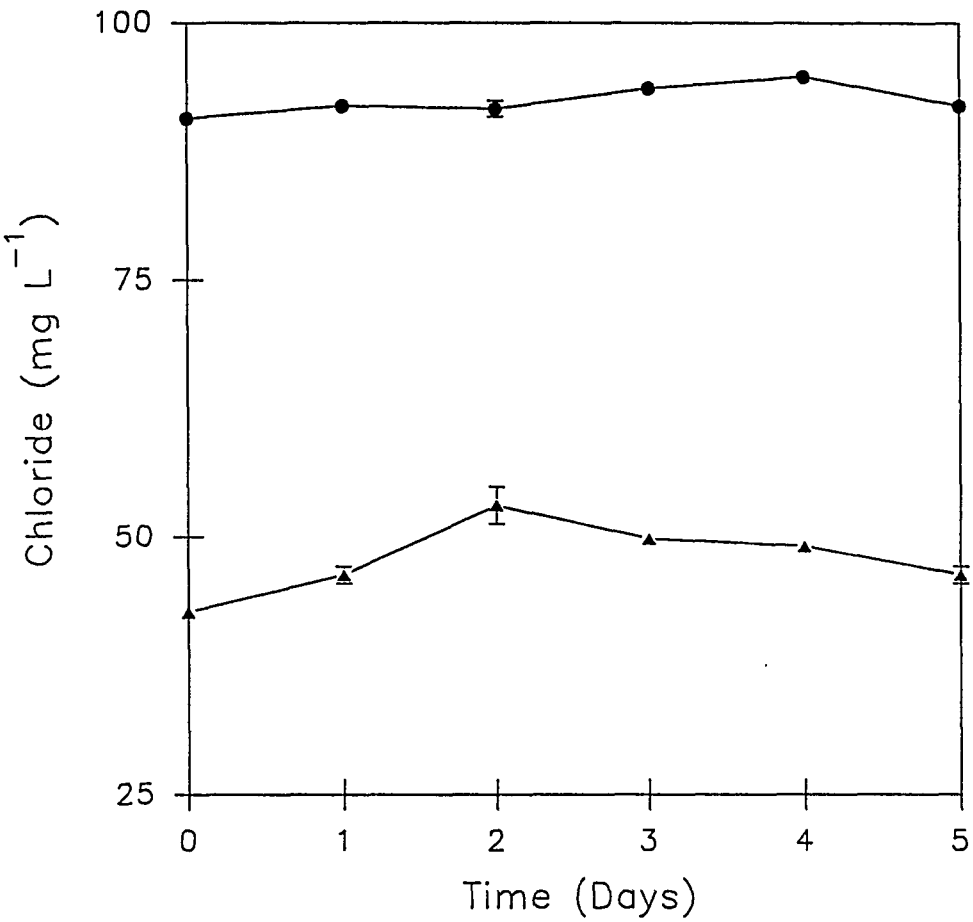

Figure 16. Chloride concentration in mesocosms (diamonds) and experimental enclosures (circles) following experimental chloride additions within experimental enclosures. Error bars indicate $\pm$ one standard error (n = 6)

Table 3. ^{15}N atom percent enrichment of plant fractions within enclosures placed *in situ* within wetland mesocosms

Fraction	Loading Pattern	Atom %	S.E.M.	n
Dead Floating	Zero	0.375	0.001	4
	Intermediate	1.234	0.247	3
	Chronic	0.667	0.049	3
Dead Standing	Zero	0.378	0.001	4
	Intermediate	0.685	0.022	3
	Chronic	0.525	0.010	3
Live New	Zero	0.377	0.002	4
	Intermediate	0.484	0.033	3
	Chronic	0.842	0.263	3
Live Old	Zero	0.375	0.001	4
	Intermediate	0.735	0.076	3
	Chronic	0.441	0.011	3
Rhizomes	Zero	0.376	0.001	4
	Intermediate	0.984	0.039	3
	Chronic	0.760	0.094	3
Rootlets	Zero	0.372	0.000	4
	Intermediate	1.128	0.031	3
	Chronic	0.766	0.041	3

the lower nitrate loading rate. The atom percent ^{15}N enrichment of total nitrogen was significantly higher in all plant fractions in treated mesocosms except for the live new cattail fraction. The ^{15}N enrichment in these same plant fractions was also significantly greater within mesocosms receiving the intermediate nitrate loading rate.

This pattern is repeated in the upper sediment fraction, with significantly greater ^{15}N enrichment in total nitrogen in treated mesocosms (Table 4). Within treated mesocosms, there was again a significantly greater enrichment in the total-N fraction in intermediately dosed mesocosms. Within the NH_4^+-N fraction, there was significantly higher enrichment in treated mesocosms, but no significant difference between treatment levels. There was also significantly greater ^{15}N enrichment in the NO_3^--N fraction in treated mesocosms, likely in residual NO_3^--N, but no significant difference between the two treatment levels.

In sediment below two cm the atom percent enrichment was much less than in the upper two cm of sediment. There was, however, still significant ^{15}N enrichment of total-N and NH_4^+-N in treated mesocosms. There was no significant difference in ^{15}N enrichment between the two treatment levels. There was also significant enrichment in the NO_3^--N fraction in treated mesocosms, likely in residual NO_3^--N, but no significant difference in enrichment in this NO_3^--N between the two treatment levels.

The atom percent enrichment of each nitrogen fraction was combined with the mass of each fraction and used to calculate a mass balance of the amount of ^{15}N remaining in each fraction within the enclosures compared to the amount of ^{15}N added. The ^{15}N derived nitrogen in the organic nitrogen fraction in microcosms containing sediment was assumed to be the difference between ^{15}N derived nitrogen in the total nitrogen fraction minus the ^{15}N derived nitrogen in the ammonium and nitrate nitrogen fractions. Nitrogen lost as denitrification was assumed to be the difference between the amount of ^{15}N added and the sum of ^{15}N recovered in all fractions.

Denitrification was confirmed to be the dominant fate of externally loaded nitrate, accounting for nearly 80 % of the $^{15}NO_3^-$ removed from the overlying water in the experimental wetland mesocosms (Figure 17). The percent of nitrate denitrified was not significantly affected by the difference in intermediate versus chronic nitrate loading.

Table 4. ^{15}N atom percent enrichment of sediment fractions within enclosures placed *in situ* within wetland mesocosms

Fraction	Loading Pattern	Atom %	S.E.M.	n
Top Two Centimeters:				
Total	Zero	0.371	0.000	4
	Intermediate	0.408	0.005	3
	Chronic	0.379	0.001	3
Ammonium	Zero	0.359	0.001	4
	Intermediate	0.979	0.179	3
	Chronic	0.573	0.012	2
Nitrate Zero	N.D.			
	Intermediate	18.191	0.298	2
	Chronic	6.330	0.951	2
Below Two Centimeters:				
Total	Zero	0.371	0.000	4
	Intermediate	0.380	0.002	3
	Chronic	0.375	0.001	3
Ammonium	Zero	0.363	0.001	4
	Intermediate	0.590	0.075	3
	Chronic	0.477	0.035	3
Nitrate Zero	N.D.			
	Intermediate	25.895	1.632	2
	Chronic	2.959	1.642	2

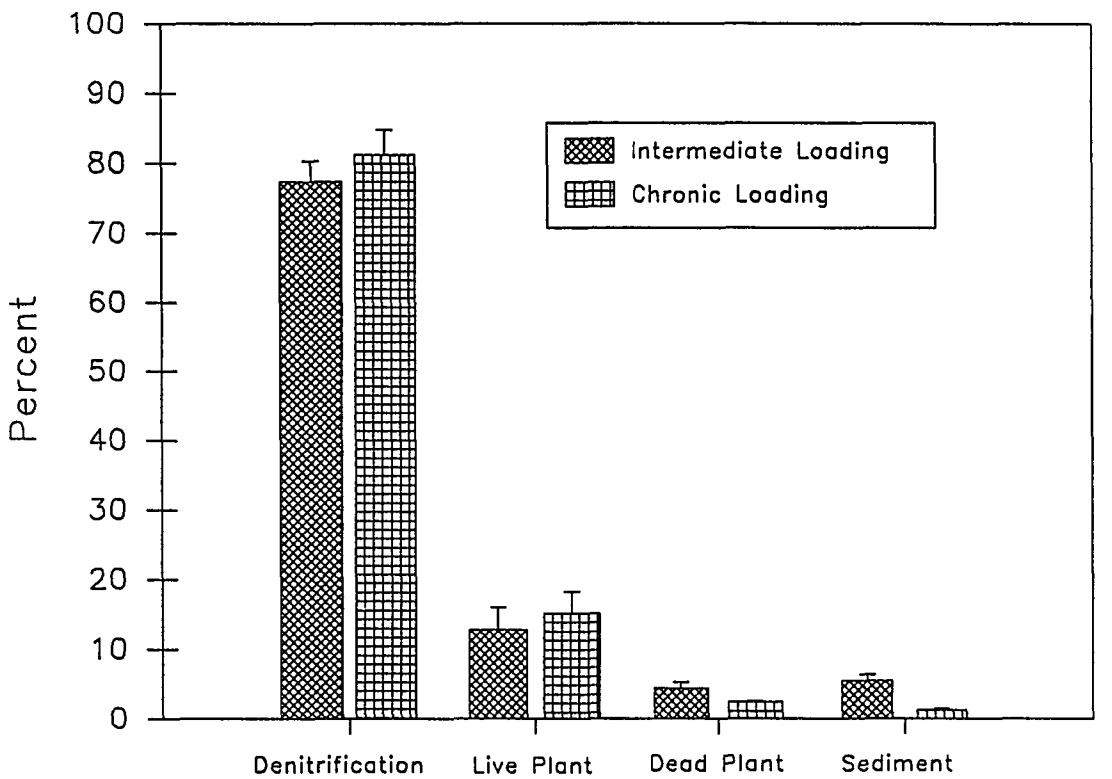

Figure 17. Fate of $^{15}NO_3^-$ following experimental addition to enclosures within intermediate and chronic dosed mesocosms. Percent is of added $^{15}NO_3^-$ transformed. Error bars indicate $\pm$ one standard error (n as in Tables 3 and 4)

Approximately 14 percent of the added $^{15}NO_3^-$ was found in the total nitrogen of the live cattail fraction, which is the sum of the live old, live new, root and rhizome fractions. There was no significant difference between nitrate loading rates in the percent of added $^{15}NO_3^-$ found within the combined live plant fraction. A treatment difference is evident within the live new cattail fraction when the live plant fractions are broken out into live old, live new, root and rhizome fractions (Figure 18). The greater biomass of live new cattails in mesocosms subjected to chronic nitrate loading resulted in a significantly greater percent of the added ^{15}N found within this fraction. As atom percent enrichments were not significantly greater in live new cattails within chronically loaded mesocosms, this difference is being driven by the greater biomass of this live new cattail cohort.

Within the total nitrogen of the dead plant fraction, which includes both dead floating and dead standing cattails, was found approximately 3.5 percent of the added ^{15}N (Figure 17). The difference between nitrate loading treatments in the percent of added ^{15}N found within the pooled dead plant fractions was not significant. When broken into the two components, however, there was a significantly greater percent of added ^{15}N found within the dead floating cattail litter within mesocosms subjected to intermediate nitrate loading (Figure 19). Most of this nitrogen demand in the dead floating litter could be attributed to assimilation and immobilization by the attached microbial community, with a greater demand within mesocosms subjected to the lower nitrate loadings.

Total nitrogen of the pooled sediment components, which includes sediment of both the top two cm and below, accounted for 5.5 percent of the added ^{15}N within mesocosms subjected to intermediate nitrate loading, significantly greater than the 1.3 percent found in sediment within mesocosms subjected to chronic nitrate loading (Figure 17). Within these combined sediment layers, very little of the added ^{15}N was found within the NO_3^--N and NH_4^+-N fractions and there was no significant difference between treatments (Figure 20). The greatest amount of ^{15}N was found in the organic-N fraction and the percent of added ^{15}N within this fraction was significantly greater in mesocosms subjected to intermediate nitrate loading. It is assumed that the majority of ^{15}N within this organic nitrogen fraction is a result of assimilation and immobilization by the microbial community at the sediment-water interface. The greater amount of added $^{15}NO_3$ immobilized to organic-N in sediments within mesocosms subjected to the lower nitrate loading again indicates a greater unmet assimilatory nitrogen demand in these systems.

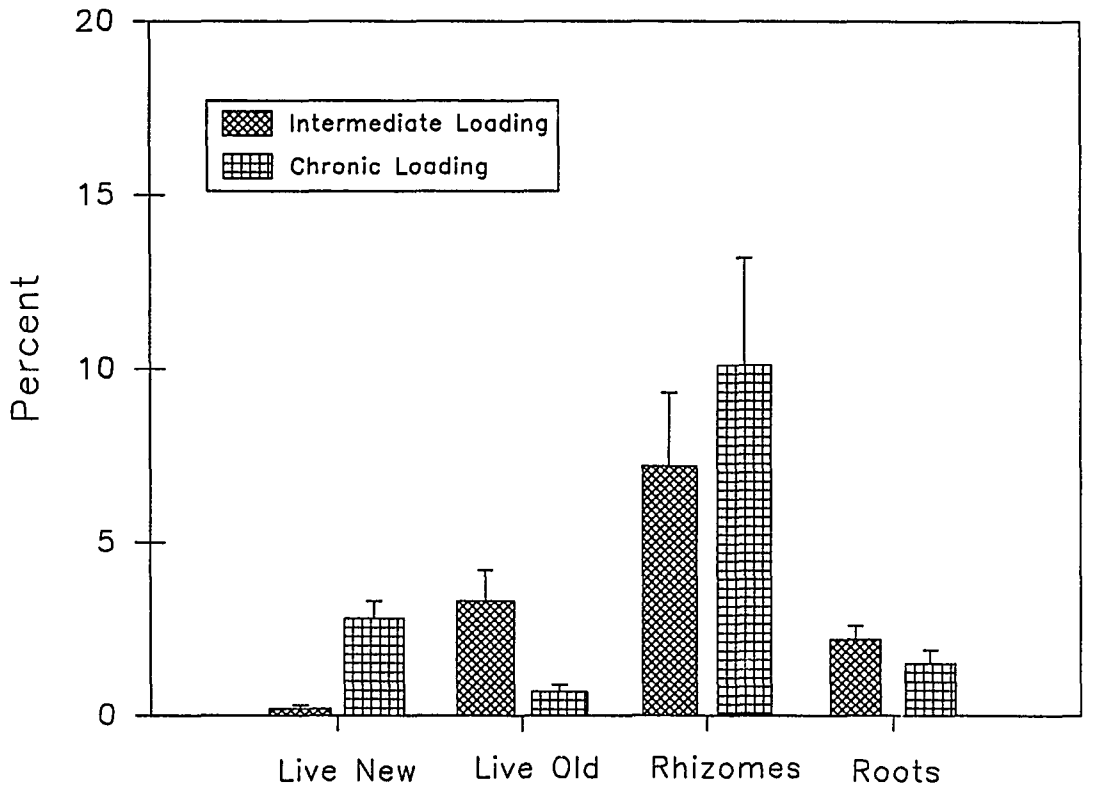

Figure 18 Percent of transformed $^{15}NO_3^-$ incorporated within live plant fractions following experimental addition to enclosures within intermediate and chronic dosed mesocosms. Error bars indicate $\pm$ one standard error (n as in Table 3)

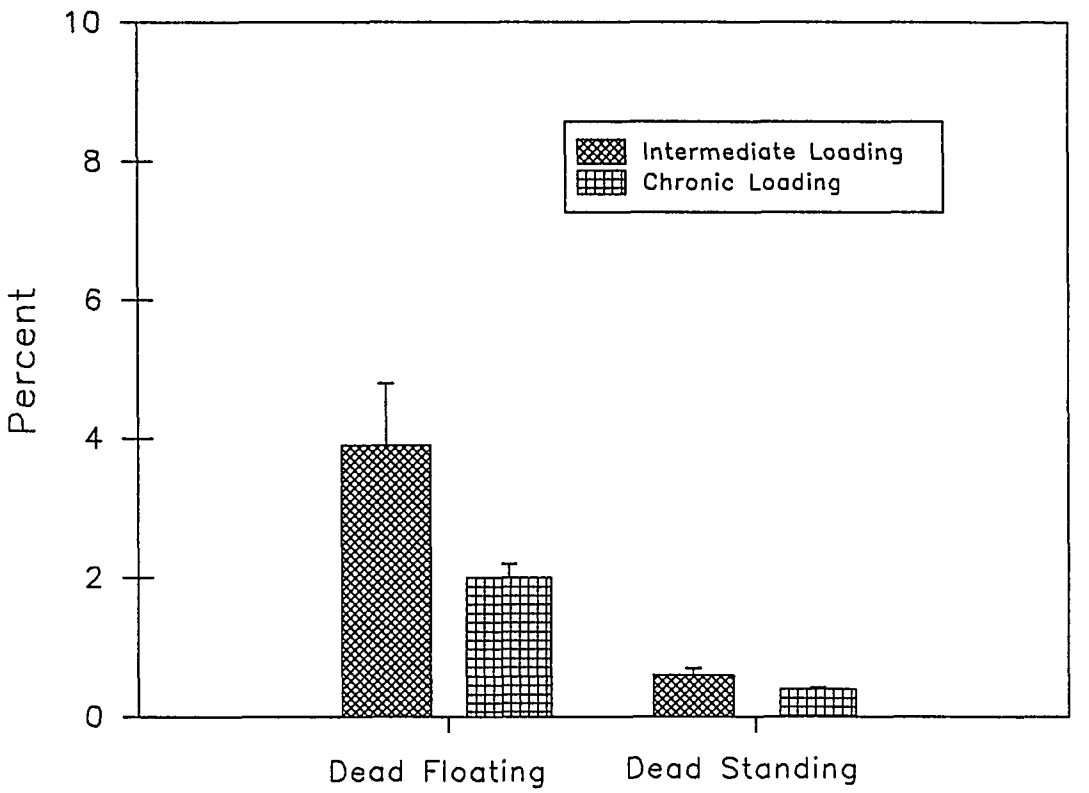

Figure 19 Percent of transformed $^{15}NO_3^-$ incorporated within dead plant fractions following experimental addition to enclosures within intermediate and chronic dosed mesocosms. Error bars indicate $\pm$ one standard error (n as in Table 3)

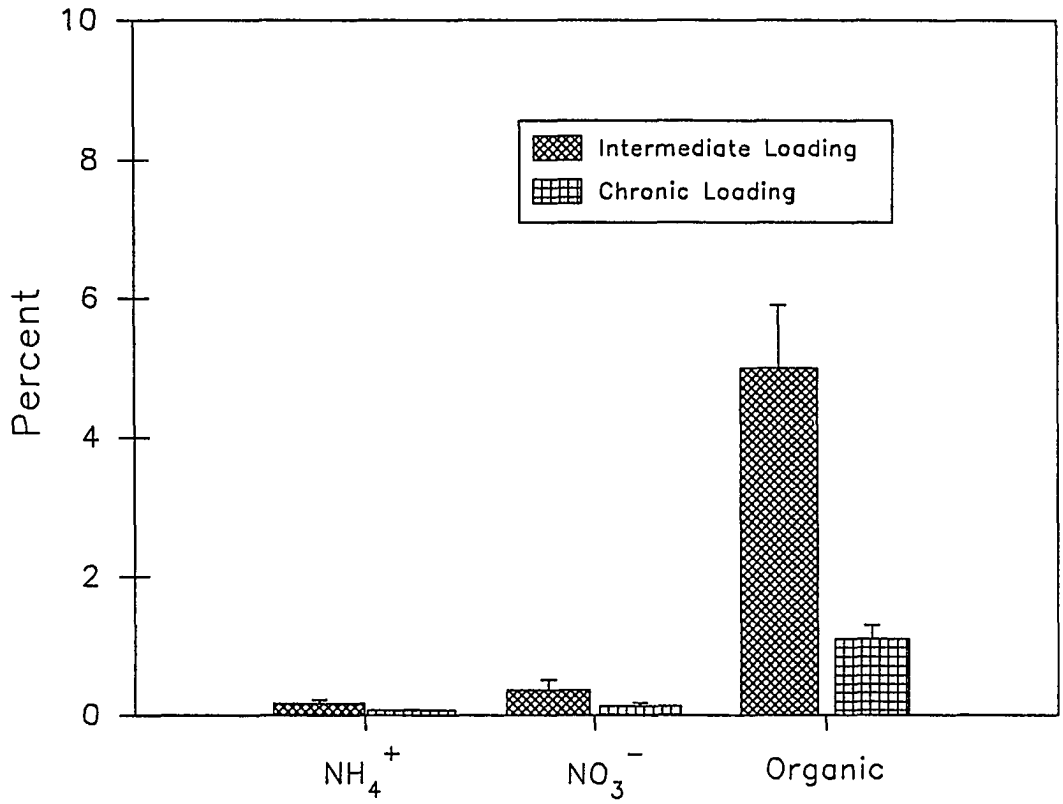

Figure 20. Percent of transformed $^{15}NO_3^-$ found within nitrogen fractions within the pooled sediment fractions following experimental addition to enclosures within intermediate and chronic dosed mesocosms. Error bars indicate $\pm$ one standard error (n as in Table 4)

Within just the top two cm of sediment, the pattern is much the same as for the pooled sediment data (Figure 21). Very little of the added ^{15}N was found within the NO_3^--N and NH_4^+-N fractions and there was no significant difference between treatments. The greatest amount of ^{15}N within the top two cm of sediment was found in the organic-N fraction and the percent of added ^{15}N within this fraction was significantly greater in mesocosms subjected to the lower nitrate loading.

Very little of the added ^{15}N was found in sediment below the top two cm (Figure 22) and there was no significant difference between loading treatments. The small amount of ^{15}N found within the sediment below two cm indicates that $^{15}NO_3^-$ was not diffusing to deeper sediments. The small amount of ^{15}N ending up as NH_4^+ in any of the sediment fractions indicates that dissimilatory nitrate reduction to ammonium did not occur to a significant extent.

In summary, studies utilizing experimental wetland mesocosms confirm the considerable capacity of northern prairie wetlands to transform externally loaded nitrate. Even under highly aerobic conditions, nitrate concentrations declined rapidly in all of the mesocosm experiments. Rates of nitrate loss observed within the experimental wetland mesocosms are among the highest reported in the literature for any wetland system (Seitzinger 1990, Johnston 1991) and rates are strongly related to the concentration of nitrate in the overlying water.

Data suggest that exposure to nitrate may affect nitrate loss rates in systems that have had little or no prior exposure to nitrate. However, following exposure to nitrate, other factors quickly become more proximate in the control of nitrate loss rate.

Denitrification was confirmed to be the dominant sink for externally loaded nitrate, accounting for nearly 80% of the $^{15}NO_3^-$ removed from the overlying water. The rest of the $^{15}NO_3^-$ removed from the overlying water was immobilized. Approximately 14% of the added $^{15}NO_3^-$ was immobilized within the various live cattail fractions and their associated microbes. The balance was immobilized in the sediment and submersed litter, most likely by microbes at the sediment-water interface and attached to decaying plant litter. Dissimilatory nitrate reduction to ammonium did not occur to a significant extent. There was no effect of chronic versus intermediate nitrate loadings on denitrification. The principal effect of chronic nitrate loading was to reduce the assimilatory nitrogen demand.

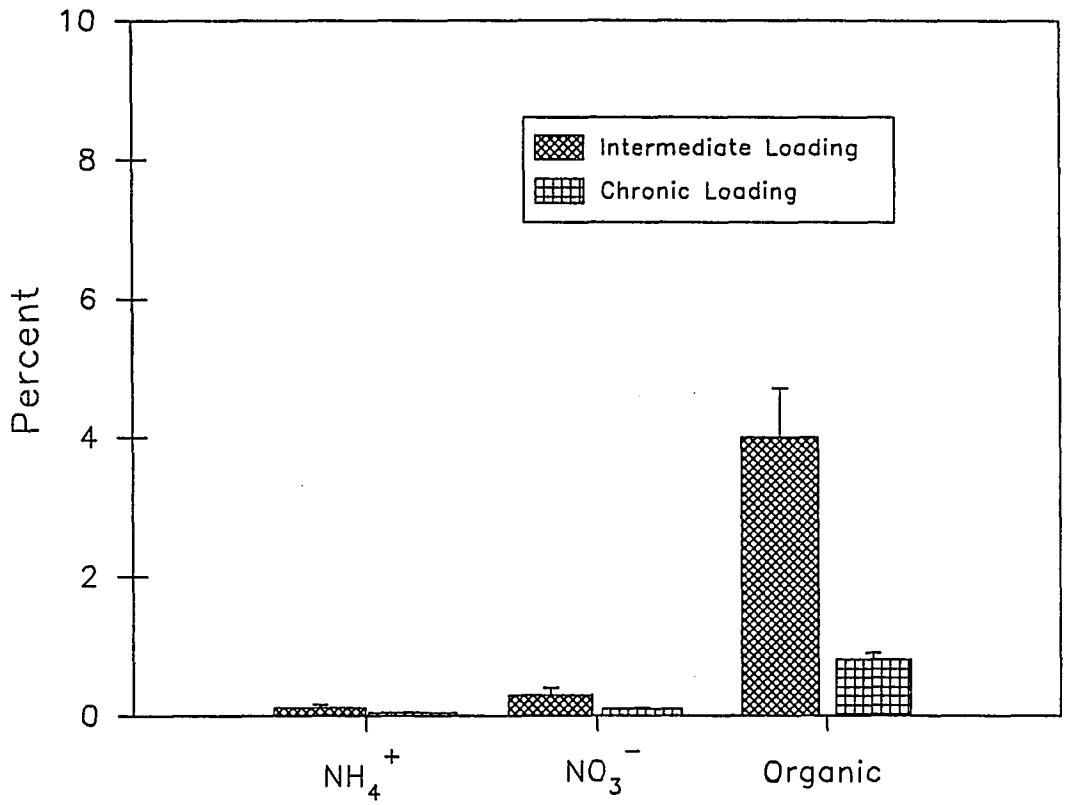

Figure 21. Percent of transformed $^{15}NO_3^-$ found within nitrogen fractions within the top two cm of sediment following experimental addition to enclosures within intermediate and chronic dosed mesocosms. Error bars indicate $\pm$ one standard error (n as in Table 4)

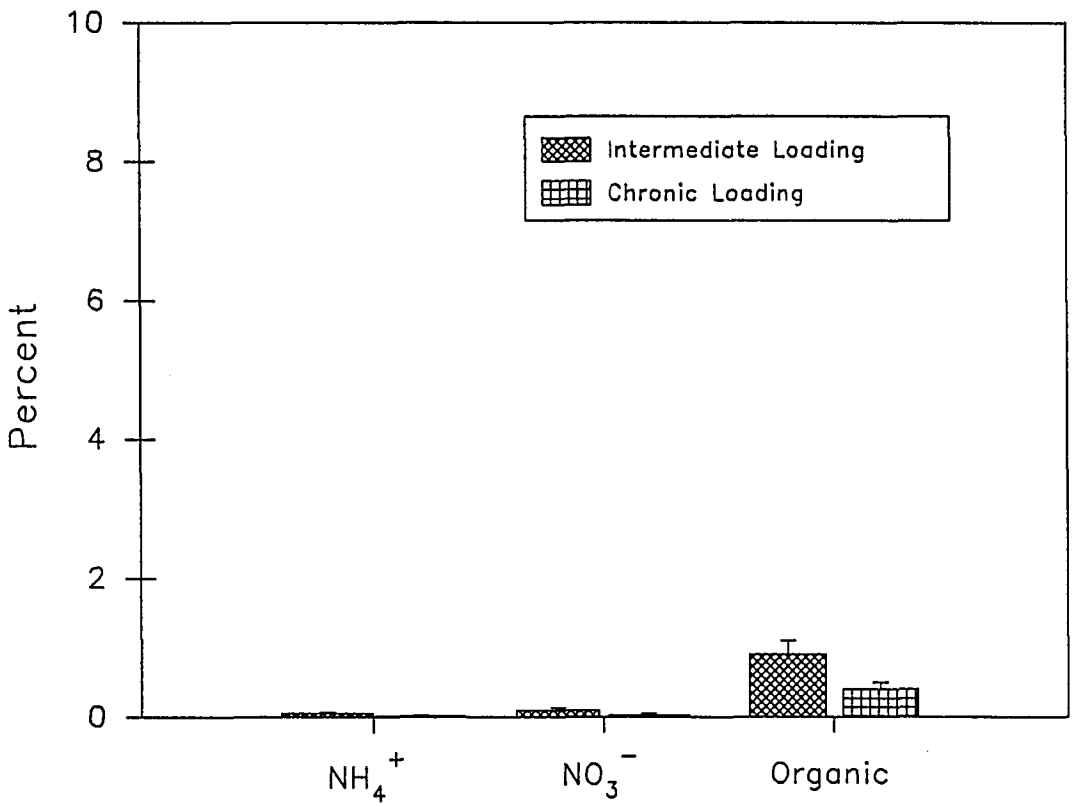

Figure 22. Percent of transformed $^{15}NO_3^-$ found within nitrogen fractions within sediment below two cm following experimental addition to enclosures within intermediate and chronic dosed mesocosms. Error bars indicate $\pm$ one standard error (n as in Table 4)

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PAPER II: THE ROLE OF PLANT LITTER IN THE TRANSFORMATION AND FATE OF NITRATE IN NORTHERN PRAIRIE WETLANDS.

INTRODUCTION

Although the importance of detritus in structuring aquatic food chains has long been realized, research to improve our understanding of the fine structure and associated biotic and resultant chemical alterations that characterizes detritus as a dynamic microenvironment has gained momentum only recently (Paerl 1984). This work has begun to stress the existence and importance of surface habitats, or microzones, as sites of altered metabolic activities among microorganisms associated with detrital particles and other surfaces (Bitton and Marshall 1980, Savage and Fletcher 1984). These observational studies of detrital microzones have laid the groundwork for more contemporary process-oriented studies designed to better define physical-chemical gradients and the resultant promotion or acceleration of specific biochemical nutrient transformation reactions known to exhibit narrow tolerance ranges to environmental variables.

It is clear that plant litter plays an important role in nutrient cycling within wetlands (Davis and van der Valk 1983, Jordan 1989). Initially, soluble nutrients are released from decomposing litter. Then, as the remaining structural components are rich in carbon and poor in nutrients, exogenous nutrients are immobilized by decomposers. Finally, as decomposition subsequently slows, mineralization gradually releases previously immobilized nutrients.

While the role of plant litter in such decomposition processes is well known, little is known about the role that detritus and decaying plant litter plays in the mediation of biogeochemical transformations of nutrients in freshwater wetlands. The principal water quality function of vegetation or decaying plant litter may be in the creation of additional environments for microbial populations (Hammer 1992). Submersed stems, leaves, and decaying litter may provide substantial quantities of surface area for attachment of microbes and constitute thin-film reactive surfaces. The importance of this function, however, has not been documented. This study examines the importance of decaying plant litter in the transformation and fate of nitrate in northern prairie wetlands.

METHODS

The role of decaying plant litter within wetlands in providing substrate for anaerobic microsites which facilitate nitrate reduction was investigated using two approaches. To establish the presence of anaerobic microzones, oxygen distribution at sediment-water and litter-water interfaces was measured using a dissolved oxygen microelectrode. In addition, the transformation and fate of nitrate in the presence and absence of litter was examined at two different scales. Enclosures placed *in situ* within wetland mesocosms provided field scale estimates and intact sediment-water microcosms collected from the wetland mesocosms allowed greater experimental control and the use of ^{15}N tracers. These studies were conducted at the Iowa State University Experimental Wetland Facility (Crumpton et al. *in press*).

Oxygen profiles of intact surficial sediments and at the cattail litter-water interface were measured within sediment-water microcosms collected in November 1991 from the experimental wetland mesocosms. Intact sediment collection procedures were as described below. Oxygen profiles were measured with a Diamond General Model 737 Clark-style microelectrode which has a tip diameter of approximately 235 um. The O_2 microelectrode was allowed to equilibrate for 0.5 hour prior to use. A micromanipulator was used to position the microelectrode, locate it at precise depths relative to the sediment or litter surface, and advance the electrode at precise intervals. The electrode output was recorded above the sediment-water or cattail litter-water interface and continuing down to the depth at which oxygen was no longer detected, or to well within the cattail litter. The position of the sediment or litter surface was determined by visible examination. Electrode current is calibrated based on electrode response (picoamps per mg O_2 L^{-1}) determined from readings at the oxygen concentration in the overlying water and at zero O_2 concentration in the anoxic sediment. Water temperatures were measured at the time profiles were taken. Duplicate oxygen profiles at the sediment-water interface were measured on 24 microcosms containing sediment. Oxygen profiles or oxygen measurements at the cattail litter surface were taken on over 30 randomly chosen pieces of litter.

Enclosures placed *in situ* within experimental wetland mesocosms were utilized to investigate the role of decaying cattail litter in the transformation and fate of nitrate. The mesocosms were constructed using uv stabilized polyethylene tanks which are 3.35 m in diameter and 90 cm deep, thus providing for approximately 9 m^2 of wetland in each

mesocosm. The tanks were filled to a depth of 60 cm with an Okoboji silty clay loam (cumulic haplaquoll), planted with cattail rhizomes (*Typha Glauca* Godr.), and flooded. A deep irrigation well supplies feedwater for the mesocosms. The mesocosms were configured with in-line fertilizer injectors which allowed for the controlled addition of desired chemicals directly into the irrigation water. Mesocosms are individually valved and water is supplied to each unit through spray nozzles around its inside circumference. Bulkhead adapters for surface drainage are located 5 cm above the sediment to prevent loss of all water in the event of a leak. Water level is maintained through the use of variable height standpipes. At the time of the enclosure study described, the mesocosms had received no nitrate addition during the preceding eight months.

The experimental enclosures placed *in situ* within the wetland mesocosms consisted of 15 cm ID plexiglass cylinders which were pushed into the sediment of the mesocosms enclosing 177 cm^2 of sediment and a water volume of approximately 6.5 L. Six enclosures were inserted into each of three mesocosms. All cattail litter was removed from the enclosures and the water was pumped out and replaced with water from control mesocosms which was amended with approximately 10 mg L^{-1} $NaNO_3^-$-N. Cattail litter approximating the amount present in each mesocosm was then added to each of three randomly chosen enclosures in each mesocosm. The other three enclosures within each mesocosm were left free of cattail litter. Each enclosure was sampled four times over the succeeding three days by collecting 20 mls of overlying water which was filtered through a 0.2 micron filter and preserved with 0.02 ml concentrated HCl. Nitrate-nitrogen was assayed using a second-derivative spectrophotometric procedure (Crumpton et al. 1992).

The role of decaying cattail litter in facilitating nitrate reduction was further investigated utilizing ^{15}N tracer techniques within sediment-water microcosms. Treatments for comparison were microcosms containing water overlying intact sediment, microcosms containing only decaying cattail litter within the water column, and microcosms containing both intact sediment and cattail litter. Controls consisted of microcosms which contained only water. There were twelve replicates for each treatment and controls. Sediment cores and cattail litter collected at the same time but not subjected to any of the treatments were used for the estimation of background ^{15}N atom percent.

Intact sediment cores were collected in November 1991 from the experimental wetland mesocosms. Polycarbonate cylinders (5.1 cm ID, 15 cm long) were pushed into

the sediment, the top closed with a rubber stopper, and the column pulled out with the intact core of sediment, and the lower end stoppered. Upon return to the laboratory, the cores were standardized to a sediment height of 5 cm. Decaying cattail litter and water for experimental addition was collected from control mesocosms at the same time as the sediment cores. The initial nitrate-nitrogen concentration of water collected from control mesocosms was less than 0.5 mg L^{-1}. Cattail litter collected was that which had been within the water column since the previous growing season.

In the two treatments which included sediment, the water overlying the cores was carefully drawn off and replaced with 130 mls of water collected from control mesocosms amended with $K^{15}NO_3$ (99.7 atom %) to an initial concentration of approximately 9 mg NO_3^--N L^{-1}. Cattail litter measured to provide approximately 200 cm^2 of surface area was then added to half of these microcosms. For the treatment which included only litter, 200 cm^2 of litter was added to 130 mls of the amended water. Controls consisted of 130 mls of the amended water in microcosms containing no sediment or decaying cattail litter.

The microcosms were incubated in the dark at room temperature (20 ^oC). Water samples were collected daily for the analysis of nitrate-nitrogen. When the nitrate-nitrogen concentration in treated cores had fallen to below 0.5 mg L^{-1} the sediment cores were sacrificed, weighed, and the sediment plus overlying water was immediately frozen at -70^oC in an ultrafreezer. The sediment plus overlying water was then lyophilized, reweighed, homogenized, subsampled, and finely ground using a mortar and pestle. Cattail litter was frozen at -70^oC in an ultrafreezer, weighed, and finely ground in a 60 mesh Wiley mill.

Determination of ^{15}N percentages was carried out in the laboratory of Dr. Alfred Blackmer of the Department of Agronomy at Iowa State University. Procedures are as described by Sanchez and Blackmer (1988). Exchangeable ammonium-N and nitrate (plus nitrite)-N contents of each sediment sample were determined by extraction with 2 N KCl and steam distillation with magnesium oxide and Devarda alloy as described by Keeney and Nelson (1982). Because distillate from these analyses were used for ^{15}N determinations, 5 ml of an ammonium nitrate standard containing 15 ug ammonium-N ml^{-1} was added to each aliquot (20 ml) of sediment extract distilled. This practice assured that each sample contained enough N to be within the working range of the mass spectrometer used for ^{15}N determinations. Distillates from the first aliquots were collected

in boric acid indicator solution and then titrated with acid as described by Keeney and Nelson (1982). Distillates from the second aliquots were collected in 2 ml of 0.08 N H_2SO_4, concentrated by evaporation of water to a volume of about 2 ml, and stored for analysis of ^{15}N. The permanganate-reduced iron modification of the Kjeldahl procedure (Bremner and Mulvaney 1982) was used to determine total nitrogen contents of sediment and plant tissue samples.

Determinations of ^{15}N in sediments, sediment extracts, and plant samples were performed by reacting the concentrated distillates with sodium hypobromite in evacuated Rittenburg flasks as described by Hauck (1982) and injecting the resulting dinitrogen gas into a Varian MAT 250 mass spectrometer. Atom percentages ^{15}N in these distillates, concentrations of ^{15}N-derived nitrate and ammonium nitrogen, and concentrations of ^{15}N-derived total nitrogen were calculated as in Sanchez and Blackmer (1988).

Statistical calculations follow Steel and Torrie (1980) and Day and Quinn (1989). Analysis of variance and orthogonal planned comparisons were used to determine significance of treatments. Differences between means were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Measurements of dissolved oxygen profiles using microelectrodes demonstrate that the cattail litter provided anaerobic microzones necessary for nitrate reduction. Both field and laboratory experiments confirmed that plant litter promoted nitrate reduction. In both cases the observed nitrate loss rate was at least twice as high in the presence of cattail litter in addition to wetland sediment. ^{15}N tracer studies demonstrate that the percent of nitrate lost through denitrification was actually greater in those microcosms containing only cattail litter versus microcosms containing only sediment.

Oxygen microprofiles at the sediment-water and cattail litter-water interfaces as measured using the oxygen microelectrodes demonstrate that anaerobic microzones necessary for denitrification occur both immediately within the sediment and at the surface of the decaying cattail litter (Figure 23). Oxygen was evenly distributed in the saturated water overlying the intact sediment core down to approximately 10 mm above the sediment-water interface at which point the oxygen concentration began to decrease. At roughly five mm above the sediment-water interface the oxygen concentration began to decrease rapidly. The gradient was steepest just above the sediment-water interface, below which the slope gradually decreased. The oxygen profile demonstrates that all the oxygen taken up by the sediment was consumed within a sediment layer only 2 mm thick.

Oxygen concentrations demonstrated a similar pattern at the interface of the water with cattail litter, with a representative profile shown in Figure 24. At approximately 2 mm from the litter-water interface the oxygen concentration began to decline very rapidly. This gradient was again steepest just above the litter-water interface, and all of the oxygen was consumed within this very thin layer with the surface of the litter being anaerobic.

Previous studies have demonstrated that variations in oxygen concentration on sediment or detritus surfaces can be very rapid and may closely follow changing light intensities if photosynthetic microalgae are present (Jorgensen and Revsbech 1985). This is demonstrated in these studies by the profile depicted in Figure 25 where the cattail litter-water interface was visibly colonized by epiphytic algae. When the oxygen profile was taken in the dark, the surface of the litter was anaerobic. When the profile of oxygen was measured in even dim light, photosynthetic oxygen production by epiphytic algae increased the oxygen concentration at the litter surface. However, even in the presence of

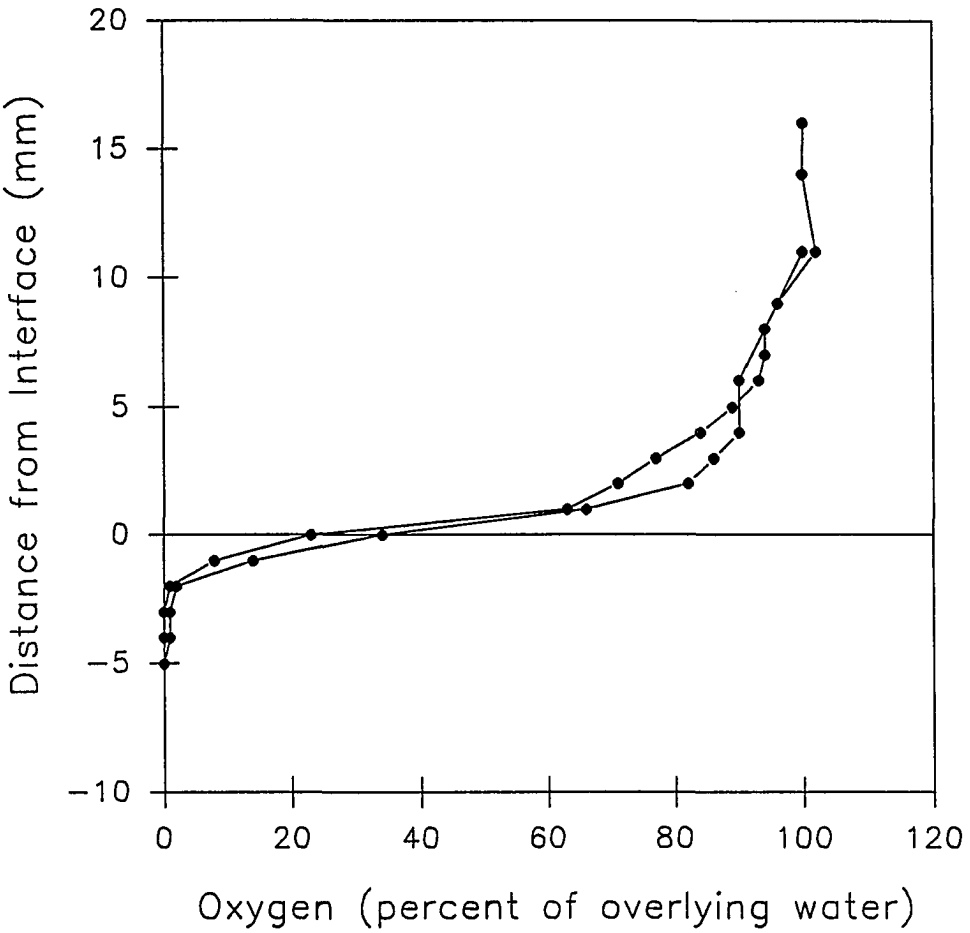

Figure 23. Replicate dissolved oxygen microprofiles measured at the sediment-water interface within a sediment-water microcosm.

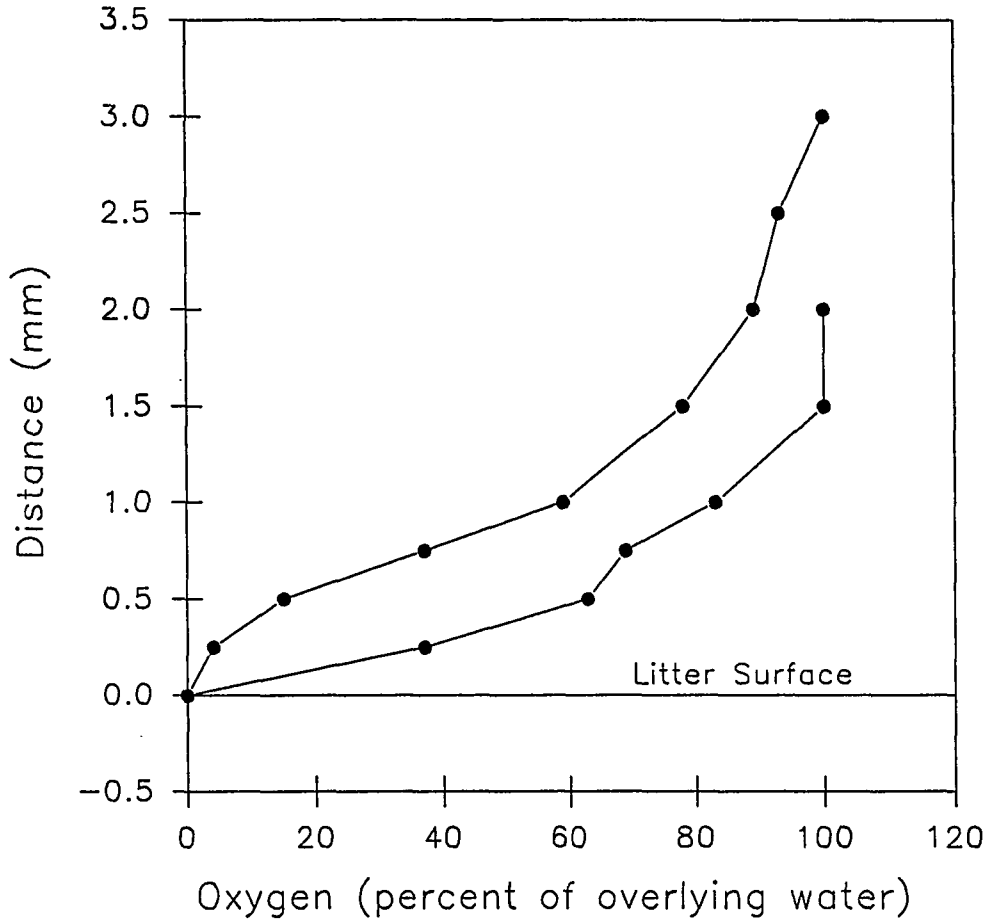

Figure 24. Replicate dissolved oxygen microprofiles measured at the litter-water interface of decaying *Typha* litter. Profiles measured in the dark

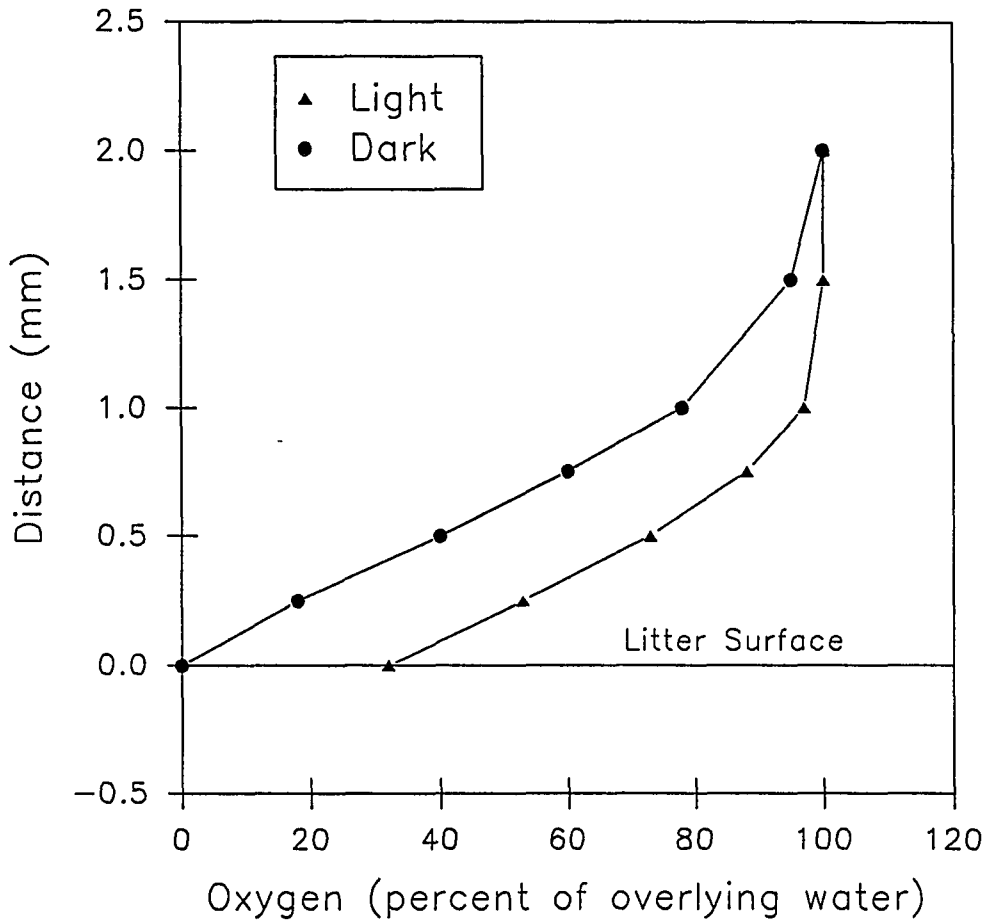

Figure 25 Dissolved oxygen microprofiles measured at the litter-water interface of decaying *Typha* litter visibly colonized by epiphytic algae. Profile measured in the light and dark

photosynthetic oxygen production at the surface, the interior of the cattail litter remained anaerobic.

Within enclosures placed *in situ* within the mesocosms, nitrate-nitrogen concentration within the overlying water declined much more rapidly in those enclosures containing the cattail litter (Figure 26). Rates of nitrate-nitrogen disappearance on a sediment area basis calculated over the first 24 hours were over 2.5 times greater in the presence of cattail litter (1.368 g N m^{-2} day^{-1}, SEM 0.29 versus 0.539 g N m^{-2} day^{-1}, SEM 0.054).

Nitrate-nitrogen concentrations in the sediment-water microcosms decreased rapidly in the presence of sediment or cattail litter, with concentrations approaching detection limits after four days of incubation (Figure 27). Among the treatments, the nitrate-nitrogen concentration declined the fastest in those microcosms which contained sediment plus cattail litter and slowest in microcosms containing only sediment. There was no significant difference in nitrate-nitrogen concentration in control cores after four days of incubation.

Treatment differences in nitrate loss rate calculated over the first day of incubation were significant, with microcosms containing sediment plus cattail litter exhibiting a nitrate loss rate nearly twice that of either sediment or cattail litter alone (Figure 28). There was no significant difference in nitrate loss rate between microcosms containing only cattail litter or only sediment.

There was substantial ^{15}N enrichment in the sediment total nitrogen and sediment ammonium fractions for both treatments containing sediment (Table 5). There was also substantial enrichment in the total nitrogen fraction of the cattail litter in both treatments in which it was included. When compared between treatments, the percent enrichment of the sediment ammonium fraction was significantly higher in those microcosms containing sediment only. Additionally, the percent enrichment in the total nitrogen fraction contained in the cattail litter was significantly higher in those microcosms containing only cattail litter compared to those microcosms containing sediment and cattail litter.

The atom percent enrichment of each nitrogen fraction was combined with the mass of each fraction to calculate a mass balance of the amount of ^{15}N remaining in each

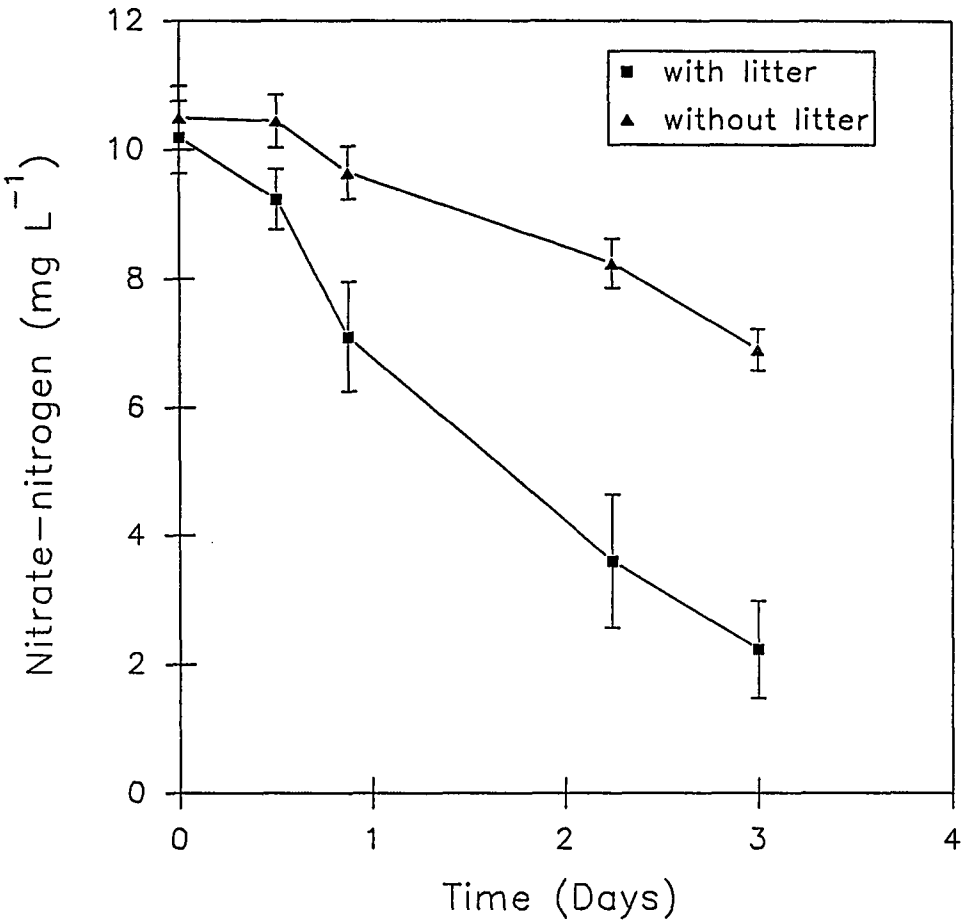

Figure 26. Nitrate-nitrogen concentrations in experimental enclosures with and without plant litter following experimental addition of nitrate. Enclosures were placed *in situ* within experimental wetland mesocosms. Error bars indicate $\pm$ one standard error (n = 9)

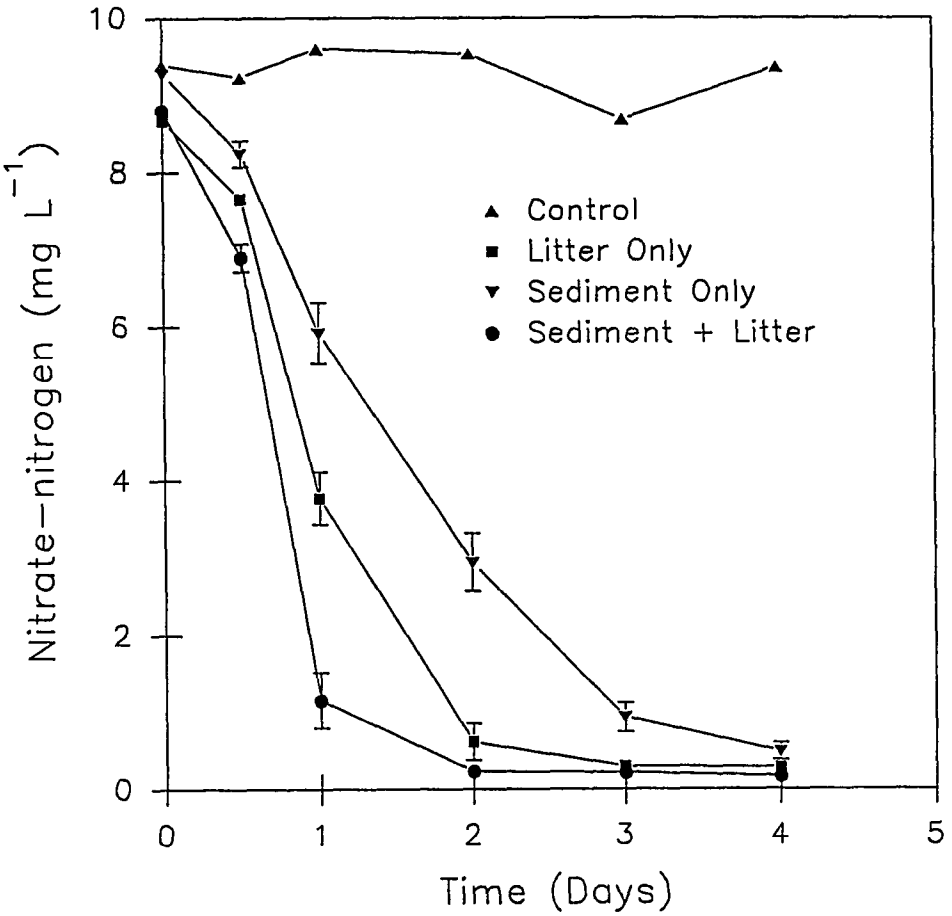

Figure 27. Nitrate-nitrogen concentrations in sediment-water microcosms with and without sediment and/or plant litter following experimental addition of nitrate. Error bars indicate $\pm$ one standard error (n = 12)

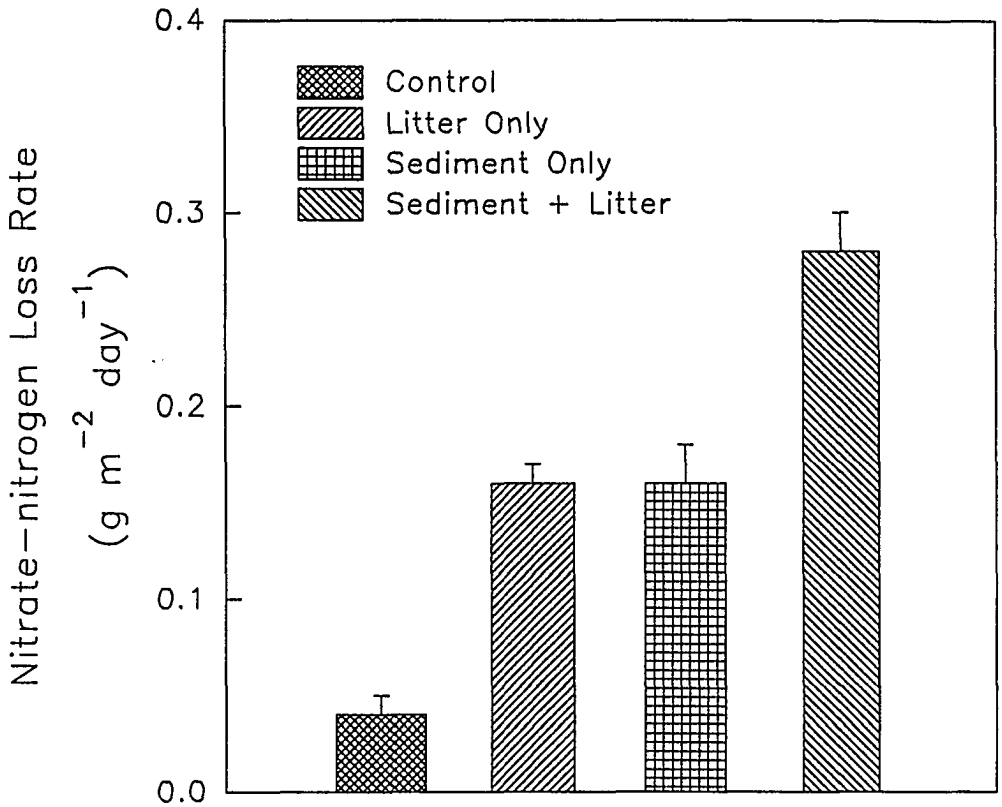

Figure 28. Nitrate-nitrogen loss rate in sediment-water microcosms with and without sediment and/or plant litter following experimental addition of nitrate. Error bars indicate $\pm$ one standard error (n = 12)

Table 5. ^{15}N atom percent enrichment of sediment nitrogen fractions and plant litter within sediment-water microcosms

Treatment	Fraction	Atom %	S.E.M.	n
Controls:	Total	0.369	0.001	4
	Ammonium	0.381	0.007	3
	Nitrate	0.382	0.011	3
	Litter (Total)	0.372	0.001	4
Sediment	Total	0.481	0.007	12
	Ammonium	4.337	0.292	12
	Nitrate	38.949	6.714	7
Sediment plus Litter	Total	0.411	0.003	12
	Ammonium	1.006	0.007	12
	Nitrate	20.898	9.058	5
	Total (Litter)	1.475	0.109	12
Litter	Total (Litter)	2.067	0.168	12

fraction compared to the amount of ^{15}N added. The ^{15}N derived nitrogen in the organic nitrogen fraction in microcosms containing sediment was assumed to be the difference between ^{15}N derived nitrogen in the total nitrogen fraction minus the ^{15}N derived nitrogen in the ammonium and nitrate nitrogen fractions. Nitrogen lost as denitrification was assumed to be the difference between the amount of ^{15}N added and the sum of ^{15}N recovered in all fractions. There was no significant difference in the grams dry weight of added cattail litter in either of the treatments in which it was included.

The ^{15}N tracer studies demonstrate that denitrification was clearly the dominant nitrate loss process within all three treatments (Figure 30) and ranged from 74.5% in microcosms containing only sediment to 81.1% in microcosms containing only cattail litter. The overall treatment effect on percent of added $^{15}NO_3^-$ lost through denitrification was significant and planned comparisons demonstrate that percent denitrification in those microcosms containing only cattail litter was significantly higher than in microcosms containing only sediment. This higher percent denitrification in microcosms containing only cattail litter versus only sediment may represent a greater competition for added $^{15}NO_3^-$ from alternate nitrate transformation processes in the sediment.

Little of the added $^{15}NO_3^-$ remained as $^{15}NO_3^-$ at the end of incubation in any of the treatments (Figure 30). Amounts ranged from 0.2% in microcosms containing sediment plus cattail litter to 1.9% in microcosms containing only sediment.

If it is assumed that the ^{15}N present as total nitrogen in the cattail litter is predominantly organic nitrogen resulting from ^{15}N assimilation and immobilization by attached microbes, then there is no significant difference between the total amount of ^{15}N remaining as organic nitrogen between any of the three treatments. The difference between treatments, however, is apparent in the partitioning of the organic nitrogen fraction, likely related to the location of the attached microbial community. In microcosms which contained only sediment, 21.5%, of the added $^{15}NO_3^-$ was present as ^{15}N labeled organic nitrogen within the sediment at the end of incubation, significantly higher than the 9.3% of added $^{15}NO_3^-$ present as ^{15}N labeled organic nitrogen within the sediment in microcosms containing sediment plus cattail litter. In these microcosms, however, an additional 12.4% of the added $^{15}NO_3^-$ was present as ^{15}N labeled total nitrogen within the cattail litter. In microcosms containing only cattail litter, 18.9% of the added $^{15}NO_3^-$ was present as ^{15}N

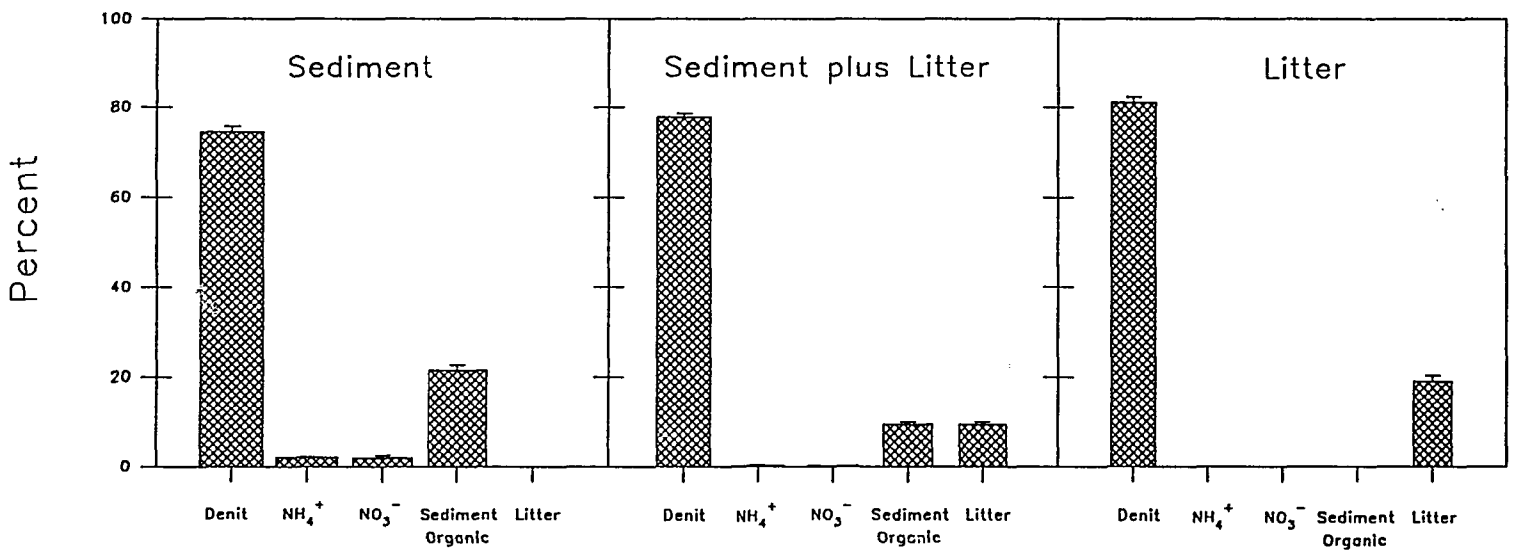

Figure 29. Fate of $^{15}NO_3^-$ following addition to sediment-water microcosms with and without sediment and/or litter. Percent is of added $^{15}NO_3^-$ transformed. Error bars indicate $\pm$ one standard error (n = 12)

labeled total nitrogen within the cattail litter at the end of incubation. This percentage was significantly greater than in microcosms containing sediment and cattail litter.

Only a very small percent of the added $^{15}NO_3^-$ was found as $^{15}NH_4^+$ at the end of incubation, ranging from 0.3% in microcosms containing sediment plus cattail litter to 2.0% in microcosms containing only sediment. This was, however, a significant difference between these two treatments. This small percent of added $^{15}NO_3^-$ present as $^{15}NH_4^+$ at the end incubation indicates that dissimilatory nitrate reduction to NH_4^+ did not occur to a notable extent.

These studies demonstrate that the primary role of decaying plant litter in the transformation and fate of nitrate in northern prairie wetlands is in providing substrate for attached microbial communities which produce anaerobic microzones that are sites for denitrification. In addition, the presence of plant litter may also increase the percent of nitrate that is lost through denitrification. Such microscale processes should also be considered for their management implications. For example, restored wetlands containing a large amount of standing vegetation and a large buildup of decaying plant litter would likely be much more efficient in nitrate removal, given sufficient contact between pollutant laden water and the substrate. Secondly, wetlands may become more efficient at removing nitrate after several growing seasons worth of litter accumulation and may likely take several years to reach a steady state with respect to their nitrate removal capacity.

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PAPER III: EFFECT OF NITRATE CONCENTRATION ON DENITRIFICATION IN NORTHERN PRAIRIE WETLANDS

INTRODUCTION

The few studies of the cycling of nitrogen in northern prairie wetlands have generally demonstrated that natural or restored wetlands can serve, at least on a seasonal basis, as nitrogen sinks (van der Valk et al. 1979, Davis et al. 1981). Denitrification is generally cited as the primary reason these wetlands may serve as nitrogen sinks. However, as Neely and Baker (1989) note, denitrification is assumed to be an important process in northern prairie wetlands based largely on circumstantial evidence; first, that conditions in the wetlands are suitable for denitrification (anaerobic conditions and a large base of organic carbon) and, second, that nitrate disappears rapidly from water overlying wetland sediments.

Wetlands within landscapes dominated by intensive row crop agriculture may frequently experience high nutrient loadings. For example, restored wetlands in the northern prairie region whose dominant water source is interrupted subsurface drainage may be exposed to very high loadings of non-point source nitrate. Numerous physical and biological factors will influence the dynamics of such nutrient loadings within these wetlands. In the case of nitrate, however, the effect of such high concentrations on its transformation and fate within northern prairie wetlands has not been documented.

Denitrification rate may be influenced by such factors as available carbon, temperature, pH, oxygen concentration, redox potential, denitrifier activity and nitrate concentration (Tiedje 1988). The question regarding the dependence of denitrification rate on nitrate concentration has received considerable debate. Some authors consider that the rate of denitrification is independent of NO_3^--N concentration (zero order) when an ample energy source is available and when the effect of nitrate diffusion is removed (Reddy et al 1978, Phillips et al. 1978, Nielsen et al. 1990). Other investigators report that denitrification follows first-order or Michaelis-Menton kinetics with respect to nitrate concentration but exhibit a very low K_m (Betlach and Tiedje 1981, Messer and Brezonik 1984). Most studies considering the dependence of denitrification rate on nitrate concentration have been conducted using terrestrial soils, sediment slurries, or pure cultures. Few studies have been conducted on the relationship between nitrate in the overlying water and denitrification rates in intact aquatic systems.

In this study, the effect of nitrate concentration in the overlying water on the rate of nitrate reduction within northern prairie wetlands was investigated using intact sediment-water microcosms. Additionally, the fate of the lost nitrate was documented using ^{15}N tracer techniques.

METHODS

Detailed studies of nitrate transformation and fate were conducted using intact sediment-water microcosms and ^{15}N tracer techniques. The objectives of these studies were to determine the fate of externally loaded nitrate within these systems and to estimate the effects of the concentration of nitrate in the overlying water on the observed rate of nitrate disappearance. In the two separate experiments described, intact sediment cores were collected from Lonnevik Marsh, a recently restored northern prairie wetland in north-central Iowa and from a subset of the experimental wetland mesocosms which had been configured as flow-through systems. A complete description of the Iowa State University Experimental Wetland Facility is given in Crumpton et al. (*in press*).

Intact sediment cores were collected from Lonnevik marsh, a restored prairie pothole wetland in Wright County, IA, in August of 1989. Polycarbonate cylinders (5.1 cm ID, 30.5 cm long) were pushed into the sediment, the top closed with a rubber stopper, and the column pulled out with the intact core of sediment, and the lower end stoppered. Upon return to the laboratory, the water overlying the cores was carefully drawn off and replaced with approximately 250 ml of water collected from the site in order to standardize initial conditions. The initial concentration of nitrate-nitrogen was less than 0.5 mg L^{-1}. Additions of $K^{15}NO_3$ (7% enrichment) were made to randomly chosen replicate cores to achieve initial concentrations of approximately 1, 3, 6, 9, and 12 mg NO_3-N L^{-1}. The cores were incubated aerobically in the dark in environmental chambers at 20 ^{o}C for 42 hours. Samples of the overlying water were collected for analysis of NO_3^--N at several times during incubation. Nitrate-nitrogen was assayed using a second-derivative spectrophotometric procedure (Crumpton et al. 1992).

Following incubation the sediment and water contained in the microcosms was sacrificed and immediately frozen at -70^{o}C in an ultrafreezer. The sediment was then lyophilized, homogenized, subsampled, and finely ground using a mortar and pestle. Determination of ^{15}N percentages was carried out in the laboratory of Dr. Alfred Blackmer of the Department of Agronomy at Iowa State University. Procedures used are as described by Sanchez and Blackmer (1988). Exchangeable ammonium-N and nitrate (plus nitrite)-N contents of each sediment sample were determined by extraction with 2 N KCl and steam distillation with magnesium oxide and Devarda alloy as described by

Keeney and Nelson (1982). Because distillates from these analyses were used for ^{15}N determinations, 5 ml of an ammonium nitrate standard containing 15 ug ammonium-N ml^{-1} was added to each aliquot (20 ml) of sediment extract distilled. This practice assured that each sample contained enough N to be within the working range of the mass spectrometer used for ^{15}N determinations. Distillates from the first aliquots were collected in boric acid indicator solution and then titrated with acid as described by Keeney and Nelson (1982). Distillates from the second aliquots were collected in 2 ml of 0.08 N H_2SO_4, concentrated by evaporation of water to a volume of about 2 ml, and stored for analysis of ^{15}N. The permanganate-reduced iron modification of the Kjeldahl procedure (Bremner and Mulvaney 1982) was used to determine total nitrogen contents of sediment samples.

Determinations of ^{15}N in sediments and sediment extracts were performed by reacting the concentrated distillates with sodium hypobromite in evacuated Rittenburg flasks as described by Hauck (1982) and injecting the resulting dinitrogen gas into a Varian MAT 250 mass spectrometer. Atom percentages ^{15}N in these distillates, concentrations of ^{15}N-derived nitrate and ammonium nitrogen, and concentrations of ^{15}N-derived total nitrogen were calculated as in Sanchez and Blackmer (1988).

In 1991 a subset of the experimental wetland mesocosms was configured as flow through systems in order to do longer term mass balance studies based on measured nitrate loading to and export from each mesocosm. ^{15}N tracer studies were performed on intact sediment cores collected from these flow-through mesocosms in September of 1991. Polycarbonate cylinders (5.1 cm ID, 30.5 cm long) were pushed into the sediment, the top closed with a rubber stopper, and the column pulled out with the intact core of sediment, and the lower end stoppered. Upon return to the laboratory, the cores were standardized to 5 cm of intact sediment and placed in a plexiglass incubation chamber connected to a thermostated, circulating water bath. The incubation chamber was filled with water which was collected at the same time as the cores from control mesocosms to a level above the top of the microcosms. The water overlying the cores was bubbled with air for a short period to circulate water and standardize conditions. The water was then drawn down to approximately 142 ml in each of the microcosms. The initial nitrate-nitrogen concentration of the overlying water was less than 0.5 mg L^{-1}. Additions of KNO_3 were made to randomly chosen replicate cores to achieve initial concentrations of approximately 3, 15

and 21 mg NO_3-N L^{-1}. Addition of $K^{15}NO_3$ (99.7% enrichment) was also made to randomly chosen replicate cores to achieve an initial concentration of approximately 9 mg $^{15}NO_3$-N L^{-1}. The cores were incubated aerobically in the dark in the thermostated, circulating water bath at 20 ^{o}C until the concentration of NO_3^--N approached detection limits (approximately 7.5 days). Samples of the overlying water were collected daily for analysis of NO_3^--N. Nitrate-nitrogen was assayed using a second-derivative spectrophotometric procedure (Crumpton et al. 1992).

Following incubation the sediment and water in those microcosms containing ^{15}N was sacrificed and immediately frozen at -70^{o}C in an ultrafreezer. The sediment was then lyophilized, homogenized, subsampled, and finely ground using a mortar and pestle. Determination of ^{15}N percentages was as described above.

Statistical calculations are as in Steel and Torrie (1980). Compared means were determined to be significantly different and $p \leq 0.05$ for analysis of variance.

RESULTS AND DISCUSSION

All results confirm the considerable capacity of northern prairie wetlands to transform nitrate. Even under highly aerobic conditions, nitrate concentrations declined rapidly in both microcosm experiments. Denitrification was the dominant nitrate loss process and rates were a function of the nitrate concentration in the overlying water over a wide range of concentrations.

Within sediment-water microcosms collected from Lonnevik Marsh, nitrate concentration in the water overlying the intact cores declined rapidly over the course of the experiment. The rates of nitrate disappearance on a sediment area basis (g m^{-2} day^{-1}) for the initial six hours of incubation versus the average concentration of NO_3^--N during this period are shown in Figure 30. There was clearly a positive relationship between observed nitrate loss rate and the nitrogen concentration in the overlying water.

The final atom percent enrichments in the NO_3^--N, NH_4^+-N, and total-N fractions of the control and 12 mg $^{15}NO_3^-$-N L^{-1} addition cores are shown in Table 6. There was significant ^{15}N enrichment in treated cores versus control cores in all three nitrogen fractions assayed.

The atom percent enrichment of each nitrogen fraction was combined with the mass of each fraction and used to calculate a mass balance of the amount of ^{15}N remaining in each fraction compared to the amount of ^{15}N added. The ^{15}N derived nitrogen in the organic nitrogen fraction was assumed to be the difference between ^{15}N derived nitrogen in the total nitrogen fraction minus the ^{15}N derived nitrogen in the ammonium and nitrate fractions. Nitrogen lost as denitrification was assumed to be the difference between the amount of ^{15}N added and the sum of ^{15}N recovered in all fractions.

Denitrification was confirmed to be the dominant loss process for externally loaded nitrate in this restored northern prairie wetland, accounting for approximately 55 percent of the $^{15}NO_3^-$ removed from the overlying water (Figure 31). Nearly 34 percent of transformed $^{15}NO_3^-$ was found in the organic N fraction within the sediment and can be interpreted to have been predominantly assimilated and immobilized by the sediment microbial community. The remaining 11 % of the transformed $^{15}NO_3^-$ was found as $^{15}NH_4^+$ and could be interpreted as $^{15}NO_3^-$ that was immobilized and subsequently

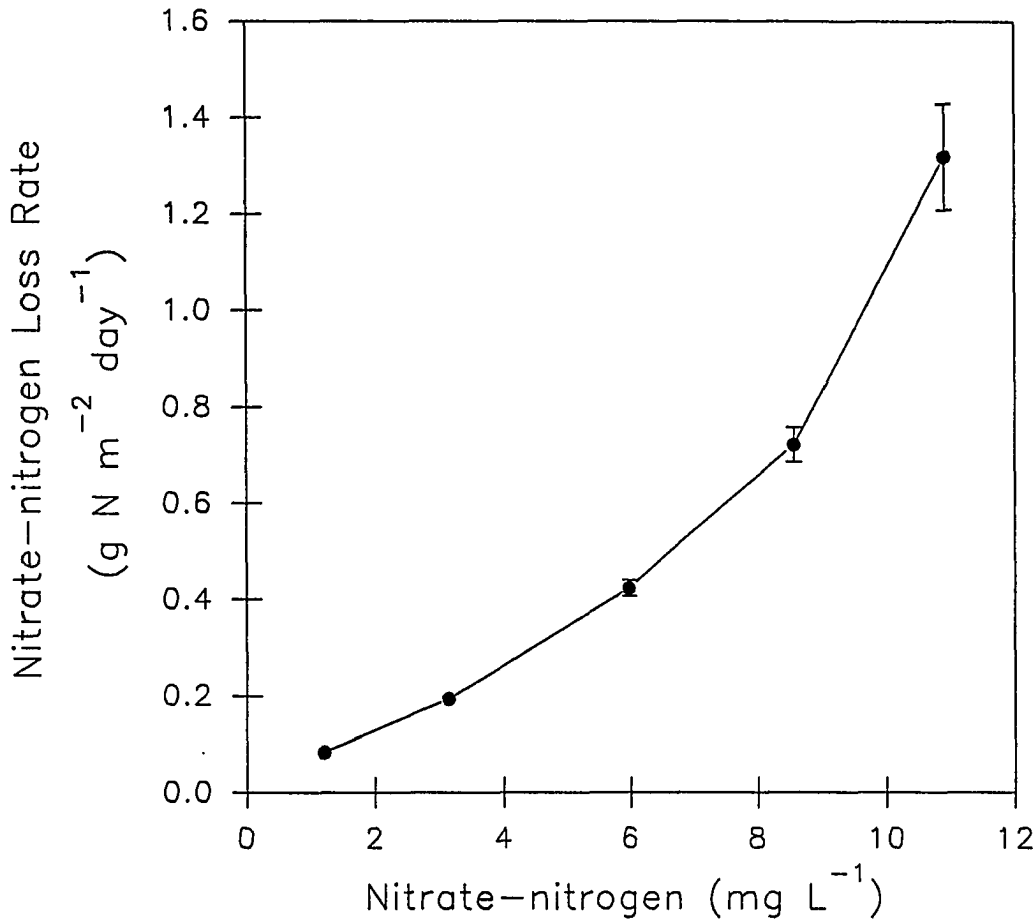

Figure 30. Nitrate-nitrogen loss versus nitrate concentration in the overlying water in sediment-water microcosms from Lonnevik Marsh. Data from the first six hours of incubation are shown. Error bars indicate $\pm$ one standard error (n = 4)

Table 6. ^{15}N atom percent enrichment of sediment fractions within sediment-water microcosms from Lonnevik Marsh

Treatment	Fraction	Atom %	S.E.M.	n
Controls:	Total	0.364	0.000	2
	Ammonium	0.361	0.000	2
	Nitrate	0.358	0.030	2
12 mg L^{-1}	Total	0.381	0.001	4
	Ammonium	0.503	0.026	4
	Nitrate	4.576	0.676	4

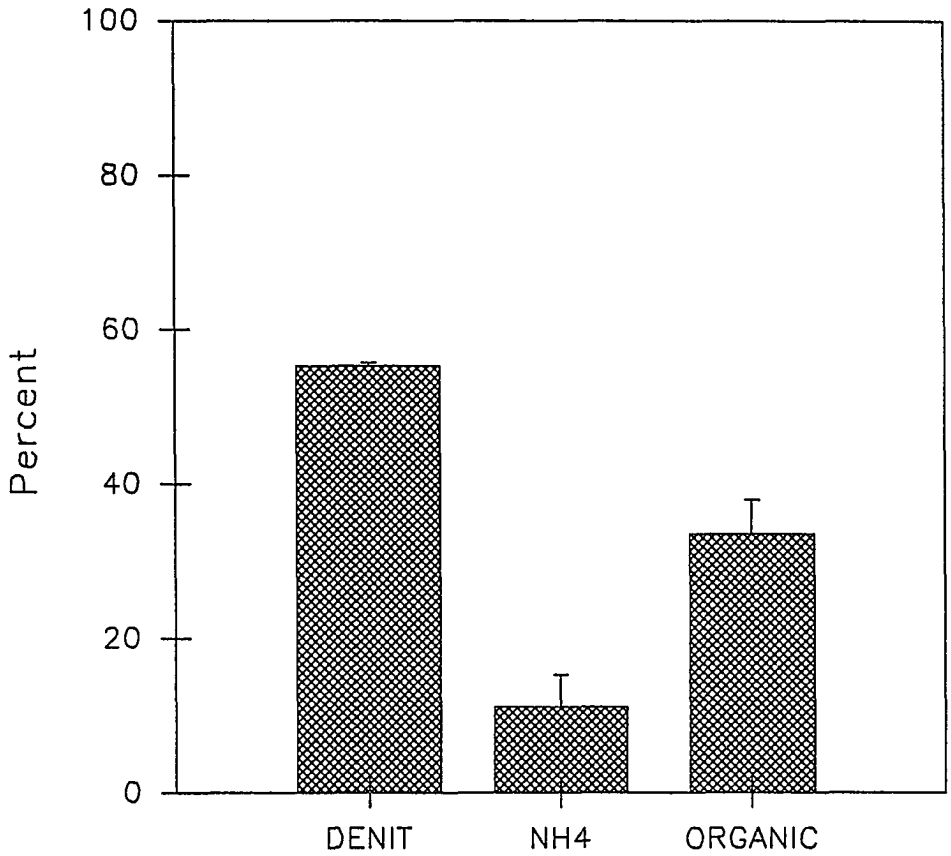

Figure 31. Fate of $^{15}NO_3^-$ following addition to sediment-water microcosms collected from Lonnevik Marsh. Percent is of added $^{15}NO_3^-$ transformed. Error bars indicate $\pm$ one standard error (n as in Table 6)

mineralized. However, given the short duration of the incubation (42 hours), it is more likely that this represents predominantly dissimilatory NO_3^- reduction to NH_4^+.

Within sediment-water microcosms collected from the wetland mesocosms, nitrate-nitrogen concentration in the water overlying the intact cores also declined rapidly for all treatments, approaching detection limits within 7.5 days of incubation (Figure 32). The rates of nitrate disappearance on a sediment area basis (g m^{-2} day^{-1}) for the initial 17 hours of incubation versus the average concentration of NO_3^--N during this period are shown in Figure 33. Once again, even with nitrate concentrations in the overlying water as high as 21 mg L^{-1} NO_3^--N, there was clearly a positive relationship between observed nitrate loss rate and the nitrogen concentration in the overlying water.

The final atom percent enrichments in the NO_3^--N, NH_4^+-N, and total-N fractions of the control and 9 mg $^{15}NO_3^-$-N L^{-1} addition cores are shown in Table 7. There was significant ^{15}N enrichment in treated cores versus control cores in both the NH_4^+-N and total-N fractions. NO_3^--N in control cores was below detection limits. Substantial enrichment was found in residual NO_3^--N in the treated cores. A mass balance of the amount of ^{15}N remaining in each fraction compared to the amount of ^{15}N added was calculated as above.

Denitrification was again deduced to be the dominant loss process for externally loaded nitrate within sediment-water microcosms from the experimental wetland mesocosms, accounting for approximately 78 percent of the $^{15}NO_3^-$ removed from the overlying water (Figure 34). Nearly 19 percent of added $^{15}NO_3^-$ was found in the organic N fraction and assumed to have been predominantly assimilated and immobilized by the sediment microbial community. Only two percent of the $^{15}NO_3^-$ added to these systems was transformed to $^{15}NH_4^+$ through either immobilization-mineralization or dissimilatory NO_3^- reduction to NH_4^+. Less than 1 percent of the added $^{15}NO_3^-$ remained as $^{15}NO_3^-$ after 7.5 days.

The observed differences in the relative fates of externally loaded $^{15}NO_3^-$ between the restored northern prairie wetland and the experimental wetlands may be related to different nitrate exposure histories. At the time of sampling, the experimental wetland mesocosms had been exposed to elevated nitrate concentrations for 45 days as part of a longer term, flow through mass balance study. The nitrate exposure history of the

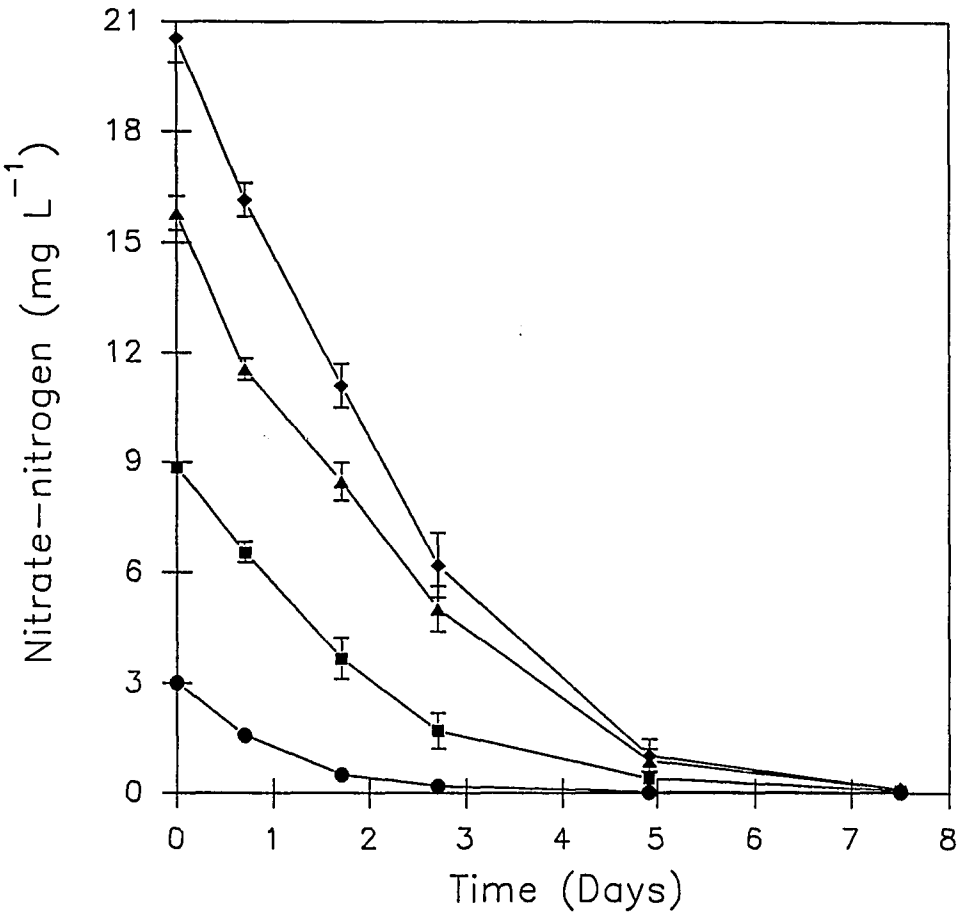

Figure 32. Nitrate-nitrogen concentration in sediment-water microcosms from flow -through mesocosms following experimental addition of nitrate. Error bars indicate $\pm$ one standard error (n= 9)

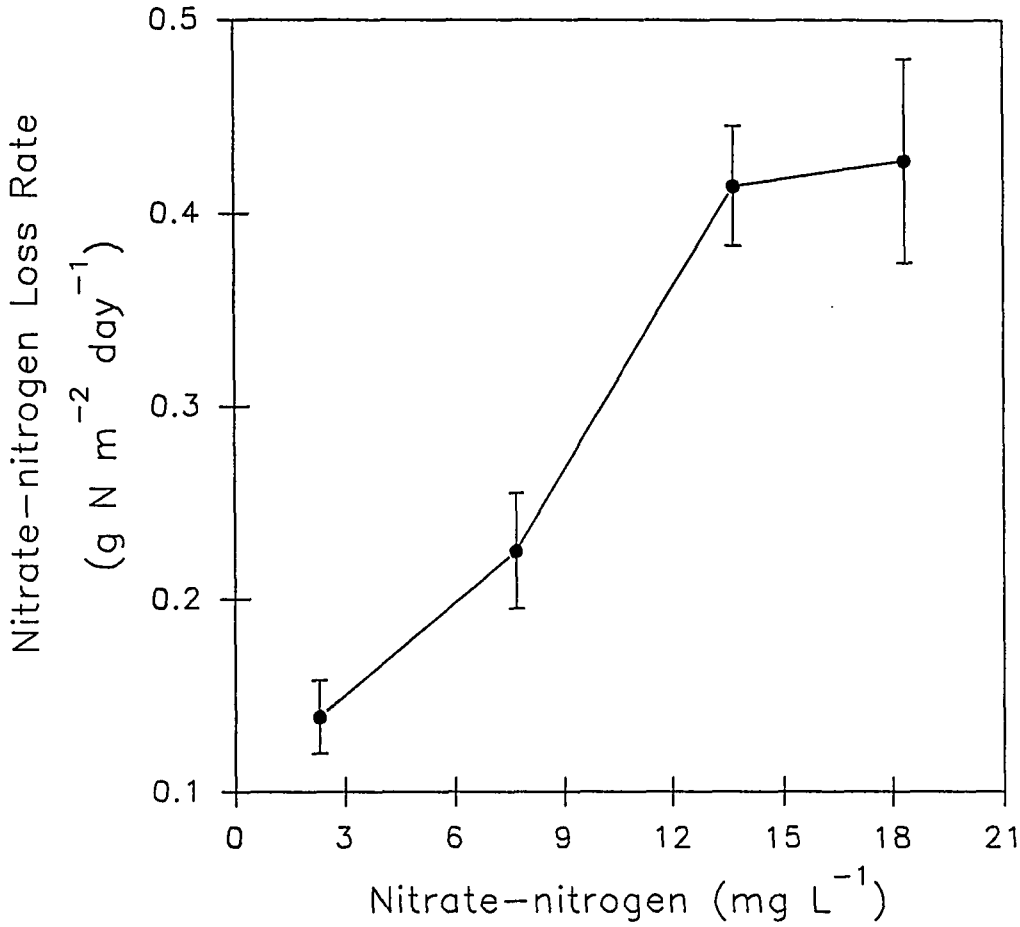

Figure 33 Nitrate-nitrogen loss versus nitrate concentration in the overlying water in sediment-water microcosms from flow-through mesocosms. Data from the first 17 hours are shown. Error bars indicate $\pm$ one standard error (n = 9)

Table 7. ^{15}N atom percent enrichment of sediment fractions within sediment-water microcosms from flow-through mesocosms

Treatment	Fraction	Atom %	S.E.M.	n
Controls:	Total	0.372	0.000	10
	Ammonium	0.372	0.001	5
	Nitrate	N.D.		
9 mg L^{-1}	Total	0.459	0.004	20
	Ammonium	3.748	0.502	10
	Nitrate	40.547	3.865	3

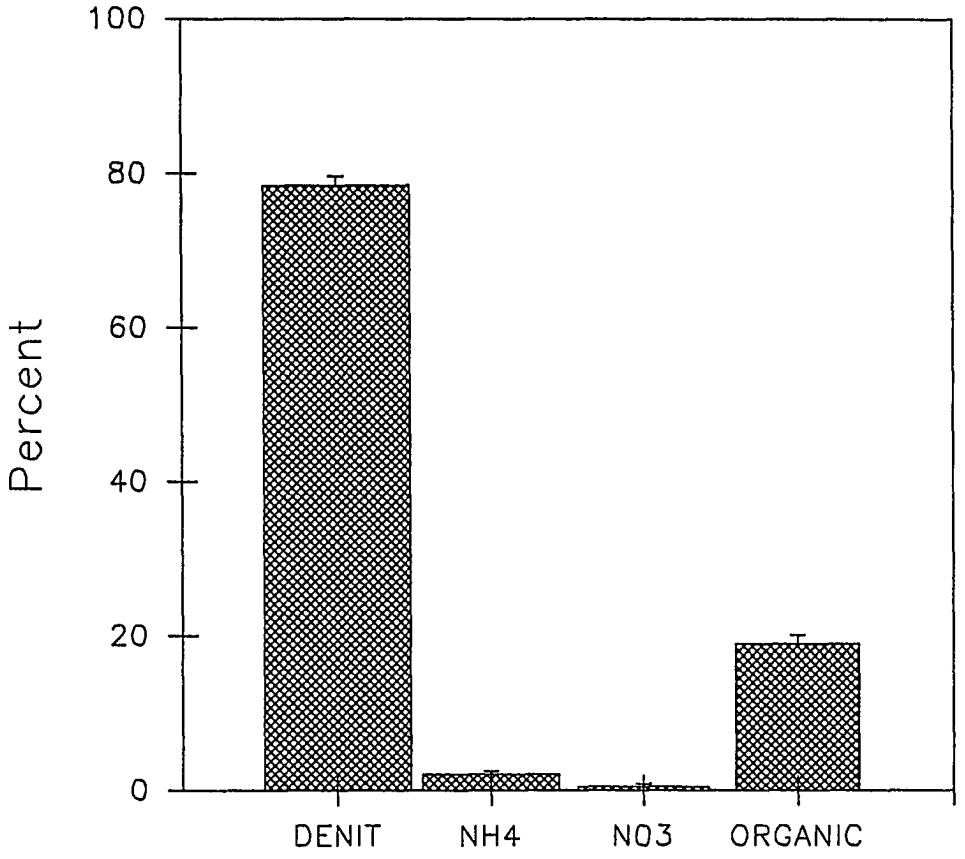

Figure 34. Fate of $^{15}NO_3^-$ following addition to sediment-water microcosms collected from flow-through mesocosms. Percent is of added $^{15}NO_3^-$ transformed. Error bars indicate $\pm$ one standard error (n as in Table 7)

sediment water systems taken from Lonnevik Marsh is less certain. However, the limited sampling that has been done at the site (data not included) indicates the sampling site within this system receives only very limited nitrate exposure. The concentration of nitrate at the site at the time of sampling, for instance, was less than 0.5 mg NO_3^--N L^{-1}.

As a result of the prior exposure to high concentrations of nitrate, the sediment water systems taken from the experimental wetland mesocosms may have a reduced assimilatory nitrogen demand (Isenhart 1992). Within Lonnevik Marsh, a larger unmet assimilatory nitrogen demand would be in direct competition with denitrifiers for added nitrate. This could account for lower percentages of added $^{15}NO_3^-$ being denitrified and a greater percentage of added $^{15}NO_3^-$ being assimilated and immobilized to organic N within this system. Alternatively, prior exposure to nitrate within the experimental wetlands may have significantly increased the activities and/or the population densities of denitrifying bacteria within these systems.

Observed nitrate loss rates within sediment-water systems from both the restored northern prairie wetland and flow-through mesocosms are clearly related to the concentration of nitrate in the overlying water over a wide range of concentrations. This is consistent with models for denitrification in agricultural streams which suggest that in the presence of high nitrate loads, denitrification rates are controlled by the nitrate concentration in the overlying water and the effective length of the diffusion path between the overlying water and the primary site of denitrification in underlying anaerobic zones (Christensen et al 1990, Nielsen et al. 1990).

These researchers suggest that the steep nitrate gradients in sediments and biofilms make it difficult to study substrate uptake kinetics of microbial mat and sediment communities as the substrate concentrations in the active layers may be quite different from the concentration in the overlying water. Their findings demonstrate that the kinetics of denitrification is zero order with respect to nitrate as the specific rate is constant with depth in the zone of denitrification. However, increases in the nitrate concentration of the overlying water will stimulate denitrification by extending the thickness of the denitrification zone with depth and not by increasing the specific activity.

In aquatic systems dominated by external nitrate loads, as is the case in many northern prairie wetlands, nitrate loss rates are apparently a function of, and may often be

limited by, the nitrate concentration in the overlying water. This is a fundamental difference between these systems and most river, lake, and coastal marine sediments where nitrate produced in the sediment from mineralization and subsequent nitrification is the major substrate for denitrification (Seitzinger 1988). Furthermore, nitrate loss rates continued to increase with increasing nitrate concentration in the overlying water, even at concentrations in excess of 20 mg NO_3^--N L^{-1}. If increases in the nitrate concentration of the overlying water stimulate denitrification by extending the thickness of the denitrification zone with depth, it seems reasonable that at some concentration this zone would be extended deep enough so that organic carbon or denitrifier activity would be limiting. In these studies conducted with sediments from northern prairie wetlands, however, this concentration was apparently not exceeded.

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GENERAL SUMMARY

Recent initiatives in wetlands restoration offer a unique opportunity to utilize restored or constructed wetlands as nutrient sinks for non-point source pollution in regions of row-crop agriculture. A lack of research on the mechanisms of nitrogen loss within freshwater wetlands, however, has inhibited the scientific community from making a credible assessment of the overall role of freshwater wetlands as sinks for non-point source nitrate.

The research presented here addressed the transformation and fate of nitrate in northern prairie wetlands. The research utilized a combination of wetland mesocosms and microcosms to conduct controlled and replicated experiments involving nitrate transformations with the overall objectives to 1) estimate the assimilative capacities of restored or natural northern prairie wetlands for nitrate, 2) determine the fate of transformed nitrate, and 3) begin to identify the factors which limit the sustained abilities of northern prairie wetlands to act as sinks for nitrate.

Results of these studies demonstrate the considerable capacity of northern prairie wetlands to transform nitrate. Even under highly aerobic conditions, nitrate concentrations decline rapidly in all of the mesocosm and microcosm experiments. In studies conducted within experimental wetland mesocosms, nitrate concentrations were consistently reduced from near 10 mg N L^{-1} to below detection limits within five days. Rates of nitrate loss on a sediment area basis often exceeded one gram NO_3^--N m^{-2} day^{-1} in the presence of several mg NO_3^--N L^{-1} and are among the highest recorded in any wetland system.

^{15}N tracer studies confirm denitrification to be the dominant fate of externally loaded nitrate in northern prairie wetlands, generally accounting for near 80 % of the $^{15}NO_3^-$ removed from the overlying water in both experimental wetland mesocosms and microcosms. The rest of the $^{15}NO_3^-$ removed from the overlying water was immobilized. Approximately 14% of the added $^{15}NO_3^-$ was immobilized within the various live cattail fractions and their associated microbes. The balance was immobilized in the sediment and submersed litter, most likely by microbes at the sediment-water interface and attached to decaying plant litter. Dissimilatory nitrate reduction to ammonium did not occur to a significant extent. There was no effect of chronic versus intermediate nitrate loadings on

denitrification. The principal effect of chronic nitrate loading was to reduce the assimilatory nitrogen demand.

Observed nitrate loss rates are clearly a function of the concentration of nitrate in the overlying water over a wide range of concentrations. This is consistent with models for denitrification in agricultural streams which suggest that in the presence of high nitrate loads, denitrification rates are controlled by the nitrate concentration in the overlying water and the effective length of the diffusion path between the overlying water and the primary site of denitrification in underlying anaerobic zones (Christensen et al 1990, Nielsen et al. 1990). Increases in the nitrate concentration of the overlying water will stimulate denitrification by increasing the nitrate diffusion gradient which results in a higher nitrate transport rate to anaerobic zones.

Cattail litter plays an important role in the transformation and fate of nitrate. Measurements of dissolved oxygen profiles using microelectrodes demonstrate that the cattail litter provided anaerobic microzones necessary for nitrate reduction. Both field and laboratory experiments confirmed the promotion of nitrate reduction by plant litter. In both cases the observed nitrate loss rate was at least twice as high in the presence of cattail litter in addition to wetland sediment. ^{15}N tracer studies demonstrate that the percent of nitrate lost through denitrification was actually greater in those microcosms containing only cattail litter versus microcosms containing only sediment.

Future research with respect to non-point source nutrients within northern prairie wetlands should focus on the continued determination of the effects of temperature, nitrate concentration, and plant litter on the transformation and fate of externally loaded nitrate in freshwater wetlands. An eventual goal should be to combine these results in the production of models of areal nitrate flux which can be combined with models of wetland hydrology and loading patterns to produce general models of nitrate loss and assimilative capacity for freshwater wetlands.

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