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Waterways Experiment Station

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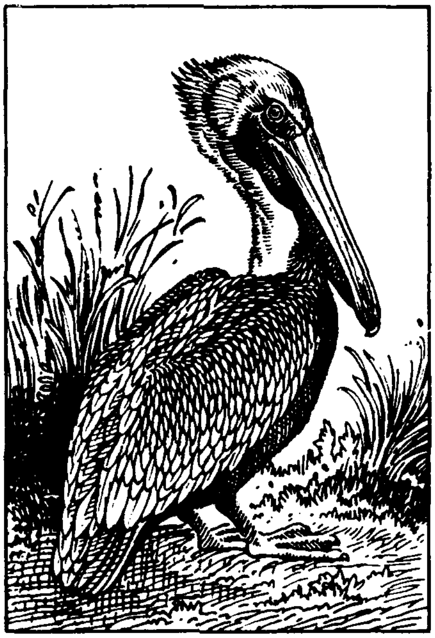
Wetlands Research Program Technical Report WRP-CP-1

Denitrification in Bottomland Hardwood Soils of the Cache River, Arkansas

by R. R. Boar, R. D. DeLaune, C. W. Lindau, W. H. Patrick, Jr.



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September 1993 – Final Report

The following two letters used as part of the number designating technical reports of research published under the Wetlands Research Program identify the area under which the report was prepared:

	Task		Task
CP	Critical Processes	RE	Restoration & Establishment
DE	Delineation & Evaluation	SM	Stewardship & Management

Corps of Engineers Research Report Summary, Sep 1993

Water Quality Processes in Bottomland Hardwoods

Denitrification in Bottomland Hardwood Soils of the Cache River, Arkansas (TR WRP-CP-1

ISSUE:

One of the most widely recognized functions of wetlands is their ability to retain or transform certain chemicals within the water, potentially improving the water quality of a body of water in and adjacent to the wetland. One of the chemical constituents of greatest importance is nitrogen. A wetland's ability to remove nitrogen from the water where it may cause eutrophication and return it to the atmosphere is dependent upon the rate at which a particular wetland performs denitrification. Denitrification rates are wetland specific and dependent upon many factors including substrate concentration, dissolved oxygen, availability of dissolved carbon, and temperature.

RESEARCH:

The primary objective of this study was to measure the rates at which nitrate is supplied and lost from nitrate-enriched river water flooding swamp sediments. These rates were estimated from the disappearance of added ^{15}N labeled nitrate and the rate at which the labeled isotope in the water column became diluted by ^{14}N produced by nitrification of ammonium in the sediment.

SUMMARY:

^{15}N labeled nitrate was added to sediment-water columns containing wetland sediment and river water to give initial nitrate-N concentrations of about 9 mg/L. Over 40 days, nitrate-N in the river water decreased by between 82 and 59 percent, which gave estimates of nitrate export from the water column of 11.5 mg N m^{-2} day^{-1} to 7.5 mg N m^{-2} day^{-1}. These values are low compared with wetland systems elsewhere and correlated directly with the organic content of surface sediments, which include the forest litter. Added glucose at least doubled rates of nitrate loss, which lead to the conclusion that the nitrogen transformations in the Cache River system may be carbon limited.

AVAILABILITY OF REPORT:

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About the Author:

The authors of this report are R. R. Boar, R. D. DeLaune, C. W. Lindau, and W. H. Patrick, Jr., who are with the Wetland Biogeochemistry Institute and the Center for Coastal Energy and Environmental Resources at Louisiana State University. Point of contact at WES is Barbara A. Kleiss, USAE Waterways Experiment Station, ATTN: CEWES-ER-W, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, Phone (601) 634-3836.

Wetlands Research Program

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Wetland Biogeochemistry Institute
Center for Coastal Energy and Environmental Resources
Louisiana State University
Baton Rouge, LA 70803

Final report

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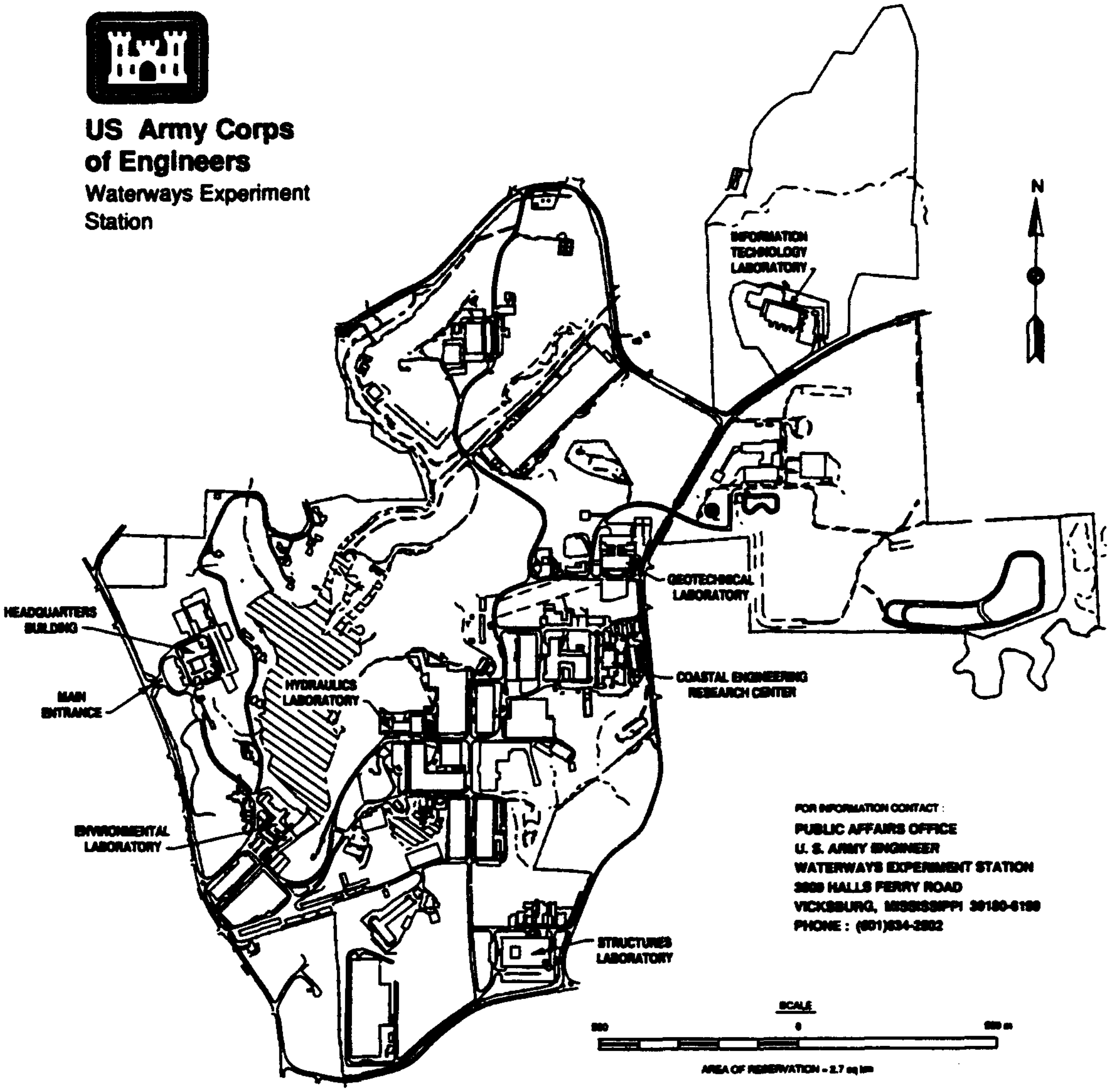

FOR INFORMATION CONTACT:
PUBLIC AFFAIRS OFFICE
U. S. ARMY ENGINEER
WATERWAYS EXPERIMENT STATION
3909 HALLS FERRY ROAD
VICKSBURG, MISSISSIPPI 39180-6199
PHONE: (601)634-2502

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Preface

The work described in this report was authorized by Headquarters, U.S. Army Corps of Engineers (HQUSACE), as part of the Critical Processes Task Area of the Wetlands Research Program (WRP). The work was performed under Work Unit 32769, "Field Investigations of Wetland Functions" for which Ms. Barbara A. Kleiss was Principal Investigator. Mr. John Bellinger (CECW-PO) was the WRP Technical Monitor for this work.

Mr. Jesse Pfeiffer, Jr. (CERD-C), was the WRP Coordinator at the Directorate of Research and Development, HQUSACE; Dr. William L. Klesch (CECW-PO) served as the WRP Technical Monitors' Representative; Dr. Russell F. Theriot, U.S. Army Engineer Waterways Experiment Station (WES), was the Wetlands Program Manager. Mr. Greg Williams (CEWES-CD-SE) was the Task Area Manager.

The work was performed at Louisiana State University. This report was prepared by Drs. R. R. Boar, R. D. DeLaune, C. W. Lindau, and W. H. Patrick, Jr., under contract No. DACW39-92-M-1638, under the direct supervision of Ms. Barbara A. Kleiss, Research Ecologist, Wetlands Branch (WB), Ecological Research Division (ERD), Environmental Laboratory (EL), WES, and under the general supervision of Mr. E. Carl Brown, Chief, WB, Dr. Conrad J. Kirby, Chief, ERD, Dr. John Keeley, Assistant Director, EL, and Dr. John Harrison, Director, EL. Dr. William D. Taylor initiated the project and collected the sediment cores. The report was reviewed by Drs. William E. Spencer and Carl F. Cerco.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

Denitrification of floodwater in swamps and shallow lakes has significant consequences for both wetland ecology and for management of water quality. This report deals with the capacity of bottomland hardwood (BLH) wetlands to reduce nitrate in floodwater from the Cache River, which drains a fertilized watershed. Denitrification, which is the biological reduction of nitrate-N to nitrous oxide and nitrogen gas, can lead to large losses of nitrogen from contaminated sediments and oxygen-depleted water.

Rates of nitrogen loss vary greatly between different ecosystems, though are usually high where there are external supplies of nitrate. Values of up to 3 mg m^{-3} h^{-1} for rates of nitrate loss through denitrification are reported for eutrophic environments by Seitzinger (1988) in a review of denitrification studies. Seitzinger concluded that while the relationship between denitrification rates and nitrogen loading seems close for estuaries, the relationship is more complicated for inland fresh waters. This may be a reflection of much larger variations between freshwater systems in the conditions that favor denitrification. Substrate concentration, dissolved oxygen, availability of dissolved organic carbon, and temperature each may limit denitrification (Beauchamp, Trevors, and Paul 1989; Fillery 1983; Knowles 1982). Even in circumstances where substrate is readily available, such as in the Cache River basin, the rate and extent of nitrate reduction may be limited by one of these other factors.

Each winter and spring, the Cache River floods large areas of forested swamp. This freshwater community is a major component of wetlands associated with the Mississippi River and its major tributaries in the southern United States. The ecological response of forested swamp to nutrient enrichment and, in the shorter term, its capacity to denitrify floodwater are relatively unstudied compared with less secluded wetland and open-water communities. Decomposition of organic matter produced within natural-forested swamps can be slow, with as much as 40 percent of the original leaf litter remaining after 46 weeks (Connor and Day 1991). These values were reported for Louisiana wetland sites where oxygen concentrations in sediment never exceeded 3 mg L^{-1} and were usually below 1 mg L^{-1}. Presumably in conditions that favor accumulation of organic matter and that are oxygen depleted, rates of denitrification will be high if a source of nitrate becomes available.

High rates of nitrate loss are desirable in rivers and wetlands receiving drainage from cultivated lowland. This may be because of direct effects of high availability of nitrate on the biological community of swamps (Boar, Crook, and Moss 1989) or because of other symptoms of eutrophication in more open waters (Moss 1990). Eutrophic effluents may supply nitrate to waterways both directly, which can be measured easily, and indirectly from organic matter that stores (and probably accumulates) past supplies. These indirect supplies are from nitrate formed by the oxidative nitrification of ammonium produced during decomposition of organic material. Since nutrient enrichment of relatively closed aquatic ecosystems leads to the inevitable accumulation of deep organic sediments, these then have the potential to contribute large amounts of nitrate to water via nitrification. Close coupling of the two pathways is thus of considerable importance in determining nitrate concentrations in water and has been shown to occur in the water column, in the sediment surface oxidized layer, and in plant rhizospheres (Jenkins and Kemp 1984; Reddy and Patrick 1984). DeLaune and Smith (1987) measured the contribution of internal ammonium pools in benthic sediment and found that the rate of conversion of ammonium to nitrate was around 70 percent of the rate of loss of nitrate through denitrification.

The aim of this study is thus to estimate both the rates at which nitrate is supplied and lost from nitrate-enriched river water flooding swamp sediments. These rates are estimated from the disappearance of added ^{15}N labeled nitrate and the rate at which labeled isotope in the water column became diluted by ^{14}N produced by nitrification of ammonium in the sediment.

2 Methods

Addition of Nitrate to Sediment-Water Columns

Twelve 15- by 25-cm cores of wetland sediment with intact plant litter were sampled in 100-cm depth polyvinyl chloride cores from three sites, B4, B7, and B8, in the forested wetlands of the basin of the Cache River in northeastern Arkansas. At the same time, approximately 200 L of river water was collected. The materials were sampled after drawdown in October 1991. Sediment cores were stored at field capacity until January 1992, when they were subsampled for analysis of organic content by loss on ignition at 350 °C. Sediment for organic analysis included the entire depth of plant litter that was present, which was variable, though no greater than 2 cm, and 1 cm of the mineral material below. During January, sediments were flooded with river water to precisely recorded depths to give an average initial floodwater volume of 9 L. Floodwater volumes adjusted to give the same head space volume of 1.325 L in each core.

Each of the 12 cores was allocated at random to one of four large containers that were filled with tap water and were intended to buffer against changes in external temperature. Three gastight 0.5-L bottles with removable screw tops were floated in each of these large outer containers. These were control bottles containing unfiltered river water.

After 10 days equilibration, cores received aliquots of 61.5 atom percent ^{15}N as KNO_3 to give final concentrations of approximately 9 mg NO_3-N L^{-1}. Actual starting concentrations of nitrate-nitrogen and of atom percent ^{15}N in solution were measured on water sampled from the 12 cores immediately after isotope addition. The cores were sealed with gastight caps and control bottles closed. All the closures were fitted with rubber septa to allow withdrawal of 10-ml head space gas and 10-ml water samples. The cores and controls were incubated at an ambient laboratory temperature that was maintained at around 22 °C.

Temperature and oxygen concentrations at 10-cm depth intervals in the sediment-water columns were measured using an Orbisphere 2607 or a YSI Model 57 oxygen probe. Measurements were made at the beginning, during the middle, and toward the end of the experiment. Samples of water and gas were taken at increasing time intervals for 12 hr and then at varying intervals over 40 days. Larger volumes of water (ranging from 0.20 to 2 L) for determination of isotopic ratio were sampled at the same times as 10-ml samples, though less frequently and only on Days 1, 12, 26, and 40.

Addition of Glucose

On Day 31, 1.04 g L^{-1} alpha-dextrose (glucose) was added to half of the replicates selected at random from each site and from controls.

Analytical Methods

Nitrate-N and ammonium-N were measured on 10-ml water samples using the steam distillation and titrametric methods of Keeney and Nelson (1982). Limits of detection are around 0.50 mg NO_3-N L^{-1} and 0.01 mg for NH_4-N L^{-1}. The method is accurate to ±0.02 percent for nitrate-N and to ±0.03 percent for ammonium-N. Duplicate distillates of larger sample volumes were combined, acidified, and evaporated down to dryness in preparation for oxidation with sodium hypobromite to liberate nitrogen gas for isotopic ratio analyses. Isotope ratios of nitrate and ammonium in water were determined with a Du Pont 21-614 mass spectrometer that is sensitive to 0.001 atom percent ^{15}N.

Nitrate added to floodwater by nitrification was calculated using the following equations:

$$P = A\,N\,(t_1) - B\,N\,(t_2)$$

where P = the production of $^{14}NO_3$-N between times t_1 and t_2 at which N_1 and N_2 were the amounts of $^{14}NO_3$ measured. The constants A and B are given by the following:

$$A = (1 - f)\,f^d \,/\, (1 - f^d)$$

$$B = (1 - f) \,/\, (1 - f^d)$$

where

$(1 - f)$ = the measured reduction of $^{15}NO_3$-N over 1 day

$d = t_2 - t_1$, which is the number of days between the measurements of N_1 and N_2 and is assumed large compared with 1 day

These equations differ from the equations given by Koike and Hattori (1978), which use changes in the isotopic ratio of ammonium as well as the ^{15}N depletion of nitrate. The equations given here do not depend on the amount of ammonium present (since this was small), nor do they rely on the assumption that dissimilatory reduction of nitrate to ammonium does not occur. Equations here are based on an exponential decay of $^{15}NO_3$ in water. An essential condition for application of these equations is that measurements of total nitrate and of $^{15}NO_3$ are made on different, and thus independent, aliquots of the same water sample.

Gas samples were analyzed for their N_2O content using a Perkin-Elmer 8410 gas chromatograph (fitted with an ECD and a Chromosorb 106 column) with working accuracy and precision of 3.7 percent or better.

3 Results

Dissolved Oxygen in the Sediment-Water Columns and Organic Content of Sediments

Surface oxygen concentrations fell from around 5 mg L^{-1} to around 2 mg L^{-1} on Day 28 in the water column above Site B4 and from 3 mg L^{-1} to below 1 mg L^{-1} in Sites B7 and B8 (Figure 1). Oxygen concentrations at the top of the water column in core B4 were always higher ($p < 0.05$) than for the other two sites, though there was generally no difference between sites in the oxygen concentrations measured at the sediment surface. Here oxygen fell to below 0.3 mg L^{-1} over 28 days. The oxygen concentration in control bottles did not fall below 4 mg O_2 L^{-1}. The organic content of sediments ranged between about 10 and 40 percent and differed between the three sites ($p < 0.001$). Values ranged from 6.88 ± 1.01 percent in Site B4 where the sediment was sandier, through 23.18 ± 1.30 percent in Site B7, to 36.89 ± 2.98 percent organic matter in Site B8 (Figure 2).

Changes in Nitrate Concentration in Water

The floodwater received amounts of nitrate-N to give starting concentrations of 9.27 ± 0.69 mg NO_3-N L^{-1}. After 40 days, Site B7 had lost 82.20 ± 3.68 percent of its initial nitrate and Site B4, where nitrate disappeared most slowly, only 59.20 ± 2.83 percent (Figure 3). Table 1 describes rates of nitrate disappearance that, after 17 days, were greater ($p < 0.01$) from cores than from control bottles that did not contain sediment. On Day 12, floodwater overlying sediment from Site B7 had lost 16.40 ± 12.90 percent of its initial nitrate-N and was the only site to differ ($p < 0.02$) from controls at this time. Controls contained 8.91 ± 0.52 mg NO_3-N L^{-1} of river water at the beginning of the experiment, and this did not change over the course of 40 days.

Water lost approximately half (B4: 45.10 ± 10.60 percent, B7: 65.35 ± 6.36 percent, B8: 58.30 ± 5.40 percent) of its initial nitrate-N by Day 30. The nitrate loss from Site B4 at this time was less than from Sites B7 and B8

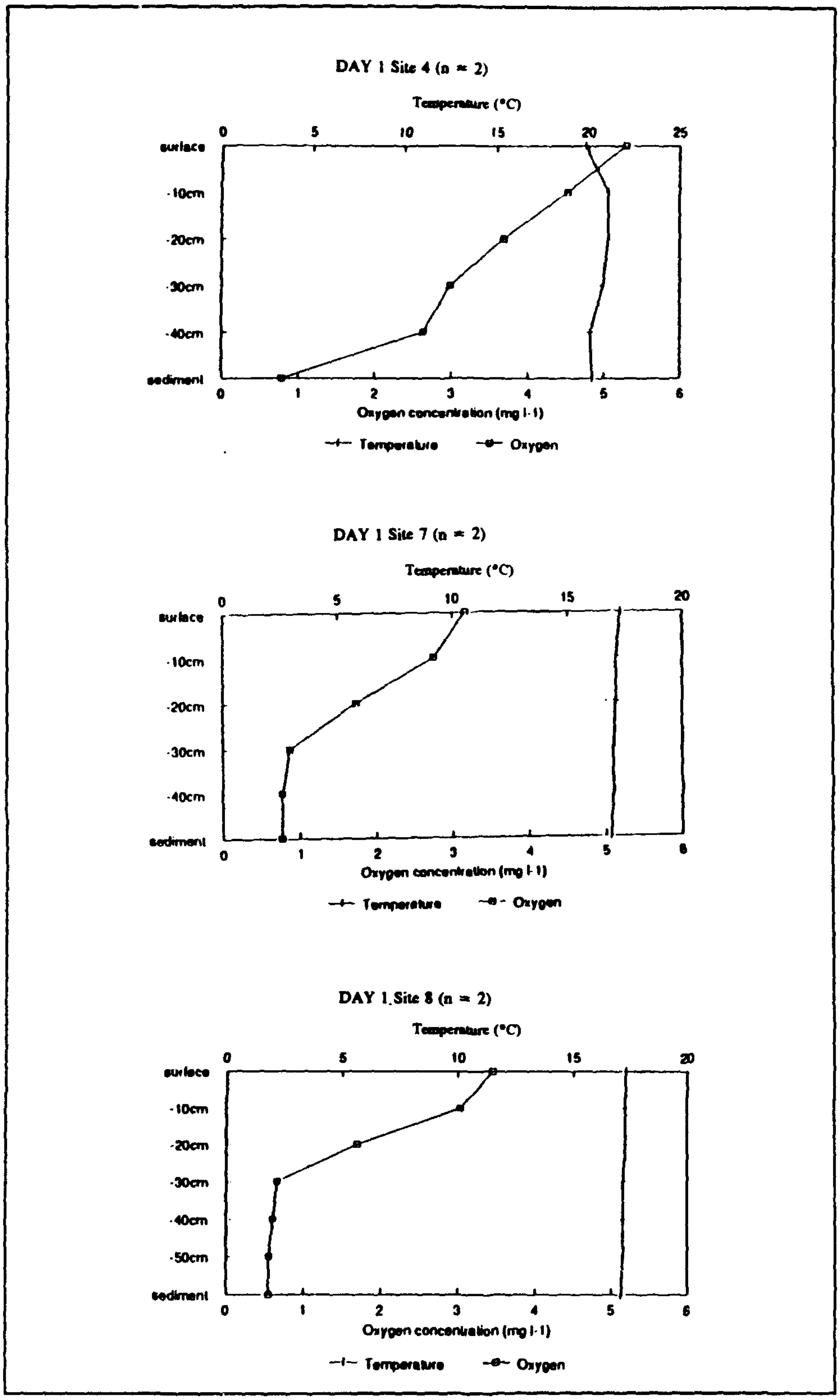


Figure 1. Oxygen and temperature profiles in sediment-water columns on Days 1, 12, and 28 (Sheet 1 of 3)

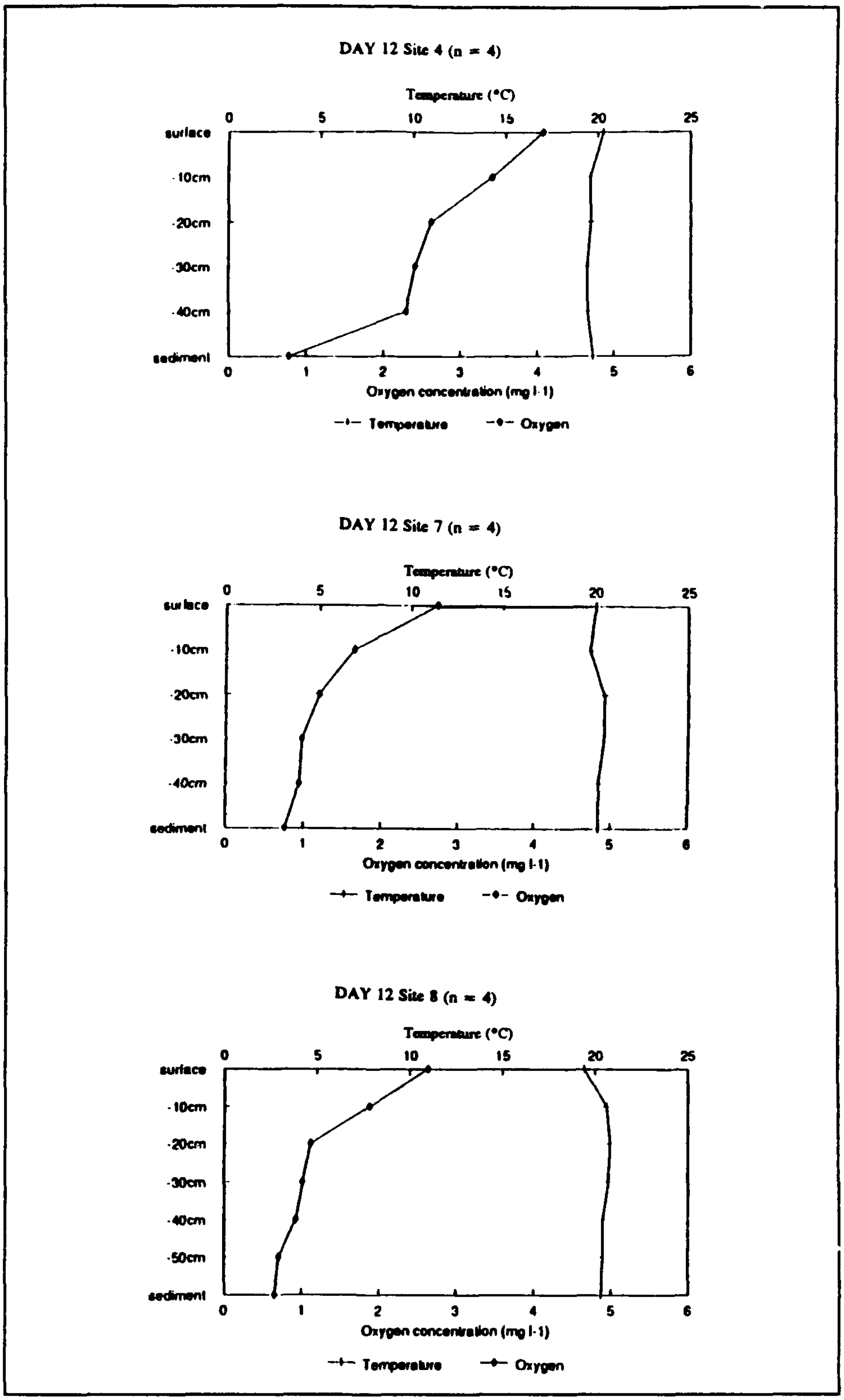
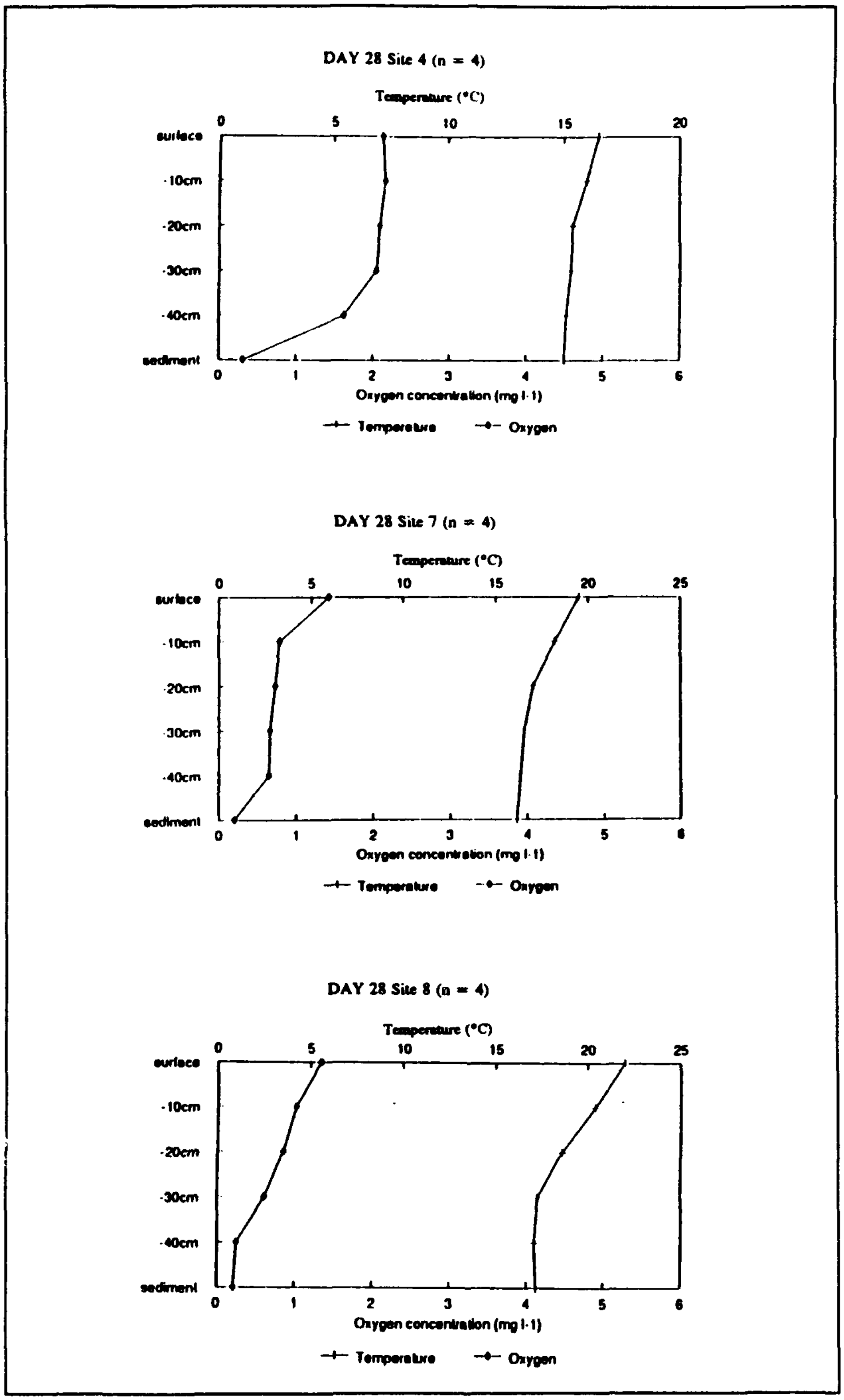


Figure 1. (Sheet 2 of 3)


DAY 28 Site 4 (n = 4)

DAY 28 Site 7 (n = 4)

DAY 28 Site 8 (n = 4)

Figure 1. (Sheet 3 of 3)

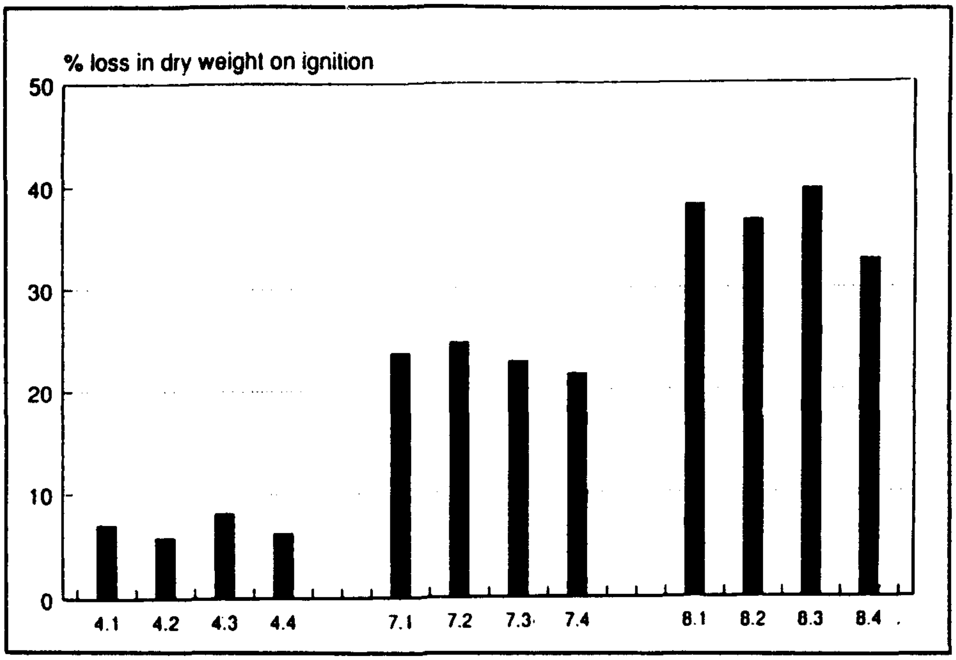


Figure 2. Initial organic content of each of the 12 cores

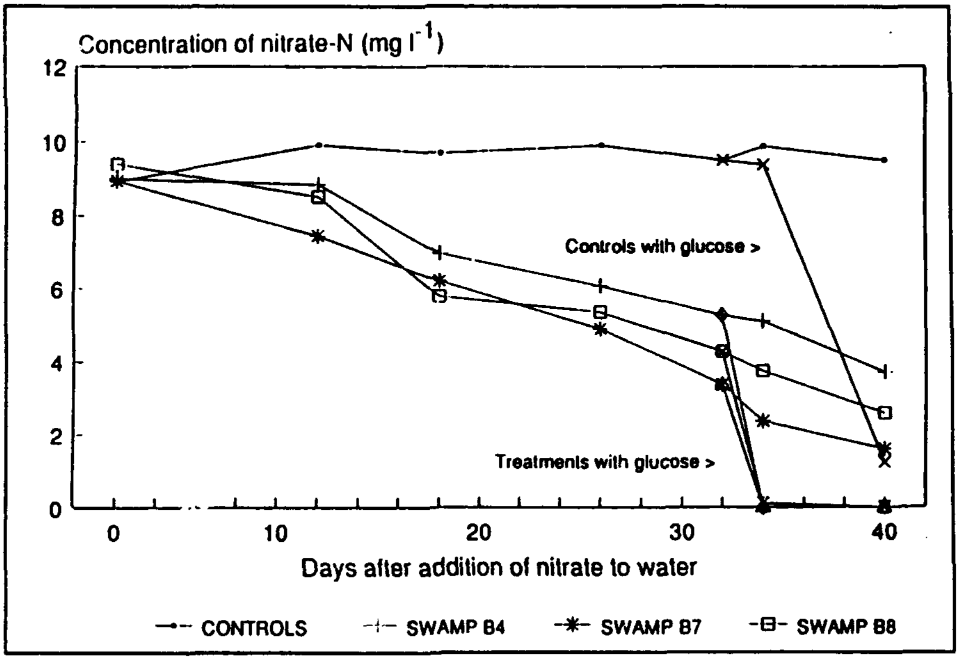


Figure 3. Changes in mean concentration of nitrate in water flooding cypress-swamp sediments and in control bottles that contain river water and no sediment (Pairs that received glucose are shown separately after Day 30)

(B7: $p < 0.001$, B8: $p < 0.05$), and there was no difference in rate of nitrate loss between B7 and B8.

The rate of loss of nitrate-N from both treatments and controls at least doubled after Day 31 when glucose was added to half of the replicates (Table 2). Within only 3 days, nitrate disappeared completely from all of the cores that received glucose (Figure 3). If the unamended cores continued to lose nitrate from the water at the average rates shown in Table 1, then nitrate-N would have reached zero around Day 69 for B4, Day 48 for B7, and Day 56 for B8. Even when glucose was added, nitrate disappearance was always more rapid ($p < 0.001$) above sediment (about 1.5 mg NO_3-N L^{-1} day^{-1}) than in control bottles (about 1.0 mg NO_3-N L^{-1} day^{-1}).

On Day 12, correlations did not exist between dissolved oxygen at any depth and nitrate concentration in the 12 cores. By Day 28, direct, though usually weak, correlations emerged between oxygen (at the sediment surface, $r = 0.47$, $p < 0.06$) and nitrate concentrations.

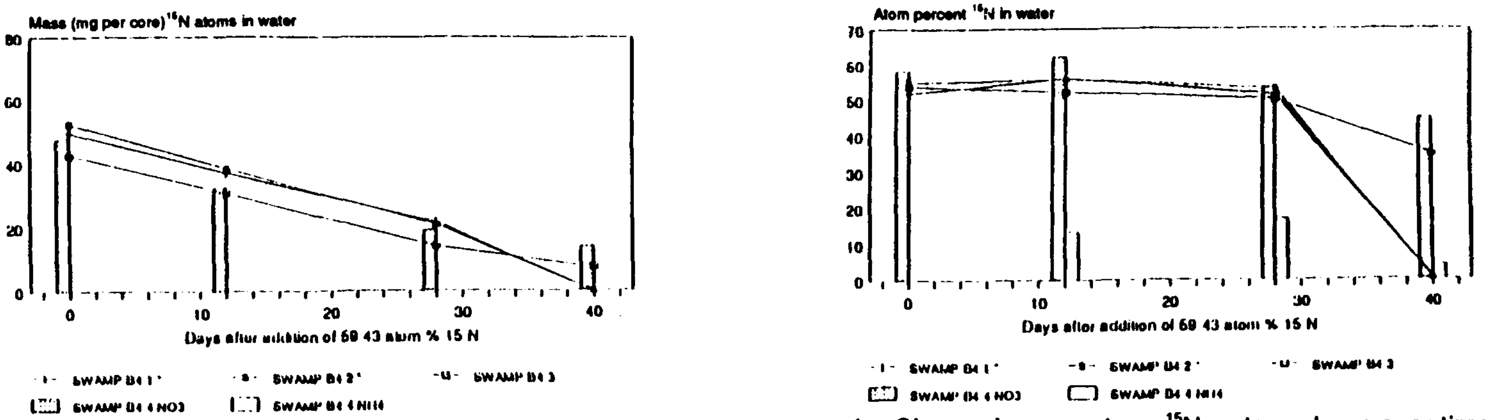
Changes in the mass of ^{15}N atoms in the dissolved inorganic nitrogen fraction of the total volume of water in each core are shown in Figure 4(a-e). Water from replicate 4 from each site was analyzed for nitrate (including nitrite) and ammonium separately. In all sites, the amount of ^{15}N labeled nitrogen in water steadily decreased (in B7, by -1.14 mg ^{15}N per core per day, $p < 0.05$; in B8, -1.19 mg ^{15}N per core per day, $p < 0.02$), with slowest disappearance from Site B4 (by -0.95 mg ^{15}N per core per day, $p < 0.01$). Glucose treatments lost all of their ^{15}N in water between Days 32 and 40. Figure 4 also shows that although the absolute mass of labeled atoms decreased, their concentration as atom percent of the total inorganic nitrogen did not.

Small amounts of ammonium that were detected in water became enriched with ^{15}N to around 12 atom percent. Isotope added to Sites B7 and B8 became more dilute over time (Figure 4(a-f)), though to only around 50 percent of initial concentrations. Rates of change in isotope ratio were low, with atom percent ^{15}N in the water from these cores decreasing significantly ($p < 0.05$) for the first time after 28 days.

Concentrations of ammonium in Sites B7 and B8 were sufficient, though always less than 0.5 mg NH_4-N L^{-1}, to yield from large sample volumes enough nitrogen for isotopic analysis. Mean atom percent ^{15}N in ammonium at the three sites was 13.19 ± 1.24 percent on Day 12 and reached a mean enrichment of 21.94 ± 9.54 percent on Day 28. By the end of the experiment on Day 40, ammonium-N was detected at low concentrations of around 0.1 mg L^{-1} and contained less than 5 atom percent ^{15}N.

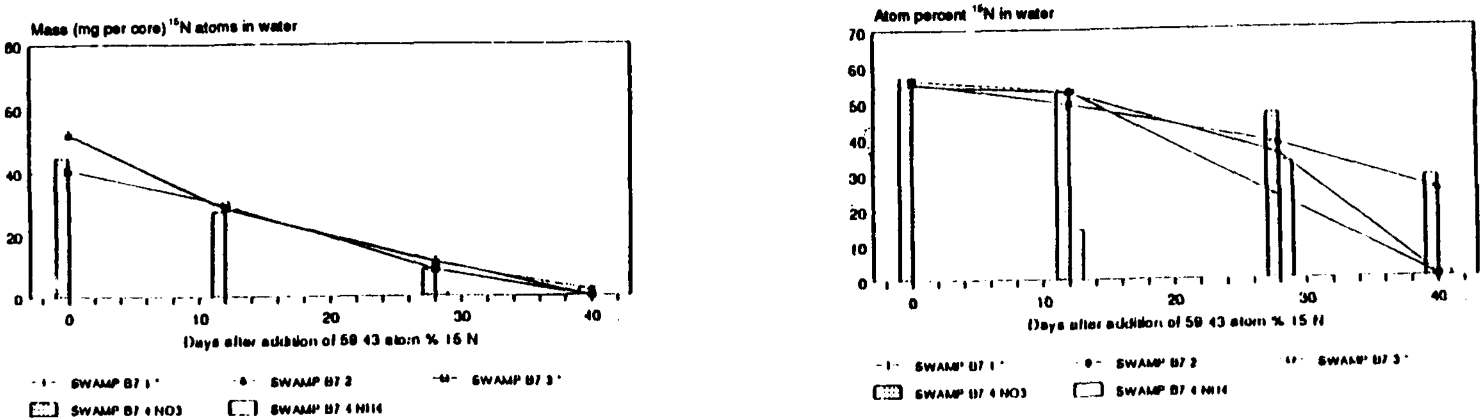
Changes in Ammonium Concentration

Ammonium-nitrogen was undetectable in river water before the addition of nitrate to cores on Day 0. After 1 day, three of the cores from Site B7 had a



a. Change in total mass of ^{15}N atoms/mg per core in water column

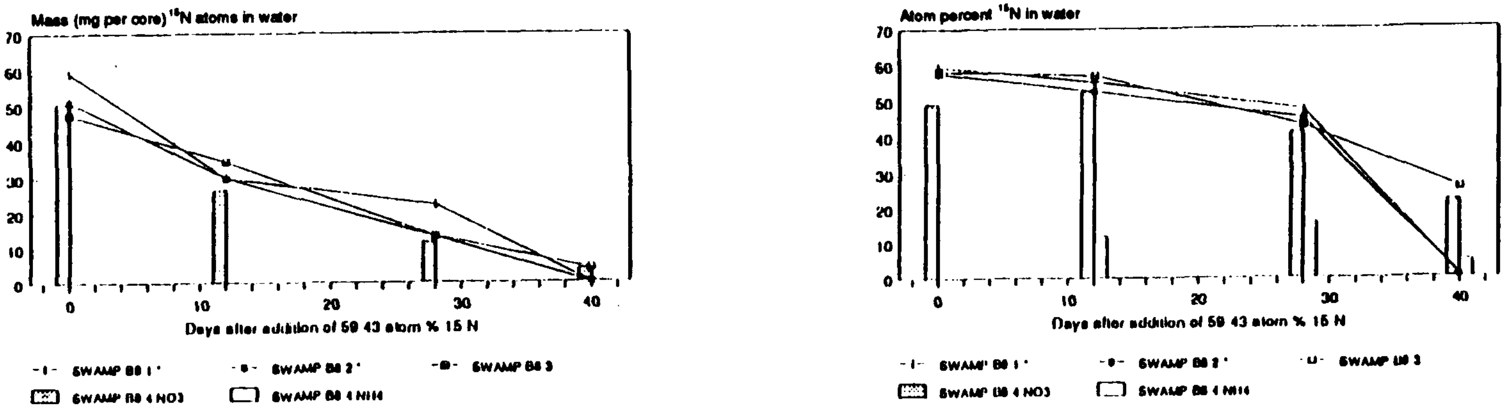
b. Change in percentage ^{15}N water column over time



c. Change in total mass of ^{15}N atoms/mg per core in water column

d. Change in percentage ^{15}N in water column over time

Figure 4. Changes in total mass of ^{15}N atoms in dissolved inorganic nitrogen in the water column of each core and changes in percentage of ^{15}N atoms in water over time. Values for replicate 4, where NO_3 (including NO_2) and NH_4 were separated in analysis, are shown by bars (Continued)



e. Change in total mass of ^{15}N atoms/mg per core in water column

f. Change in percentage ^{15}N in water column over time

Figure 4. (Concluded)

mean concentration of 0.26 ± 0.11 mg NH_4-N L^{-1} in the water column, which was similar to the amount measured in one of the replicates from Site B8. Variations in the mean concentrations of ammonium from the three sites and in control bottles are shown in Figure 5(a). Ammonium appeared to accumulate in the water overlying sediments from Sites B7 and B8 until around Day 12, not until 8 days later in control bottles containing river water, and not at all in water that flooded Site B4. Although significant differences existed between Sites B4 ($p < 0.05$) and B8 ($p < 0.05$) and controls, a difference did not occur between the mean of B7 and the control bottles. Within-site variations in ammonium were more marked for site B7 than for the other two sites (Figure 5(c)). A pattern of small increases in ammonium concentration until around Day 20 was, however, a feature of all sites.

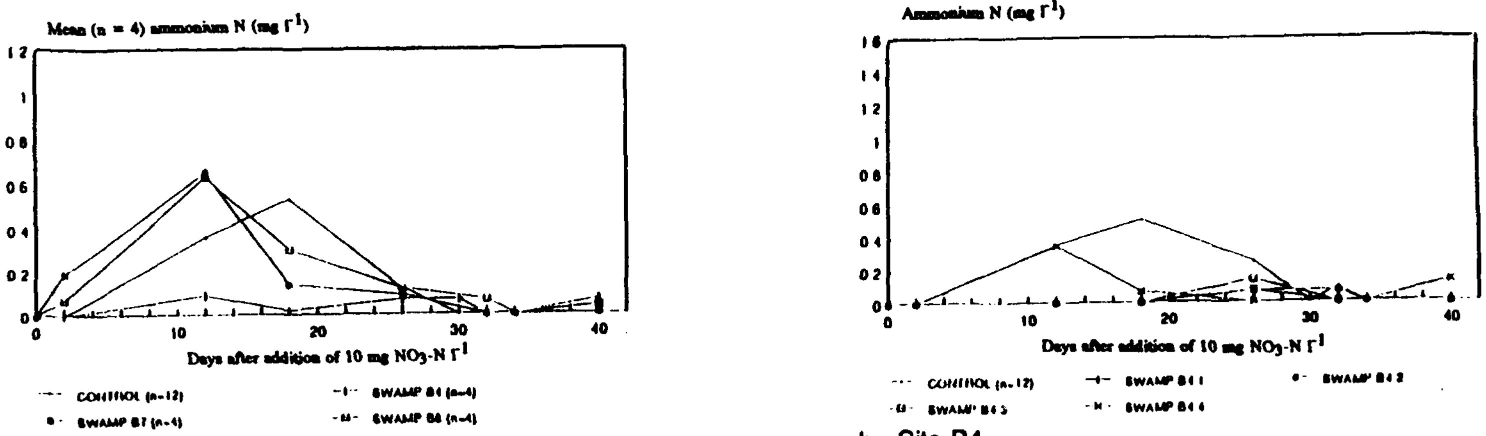
Inverse correlations ($p < 0.05$) occurred between oxygen and ammonium on Day 12, though they did not vary together on Day 28. Variations of oxygen concentration with ammonium occurred at points that were relatively high in the water column and not at the sediment surface. The high ammonium concentrations on Day 12 were thus correlated with oxygen concentrations when these were less than about 2.5 mg L^{-1} and 20 cm above the surface of the sediment.

Production of Nitrous Oxide

Rates of N_2O production are expressed as nitrogen fluxes between water and air in units of milligrams of N_2O-N evolved per square meter of sediment per day (Figure 6). The rate of N_2O evolution varied between cores (Table 3). The largest N_2O fluxes were from B8 and the lowest from B4. At the time of maximum loss of nitrogen as N_2O for site B4, which occurred on Day 17, the absolute nitrogen losses as N_2O were approximately 15 to 20 percent of those from the other two sites. The relationship between change in nitrate concentration over all 12 cores and amounts of N_2O evolved per core was not close ($r = 0.4541$, $p < 0.001$). Evolution of N_2O corresponded with 20.6 percent of the variation in nitrate concentration over the 40 days and with 37.9 percent over the first 17 days after flooding.

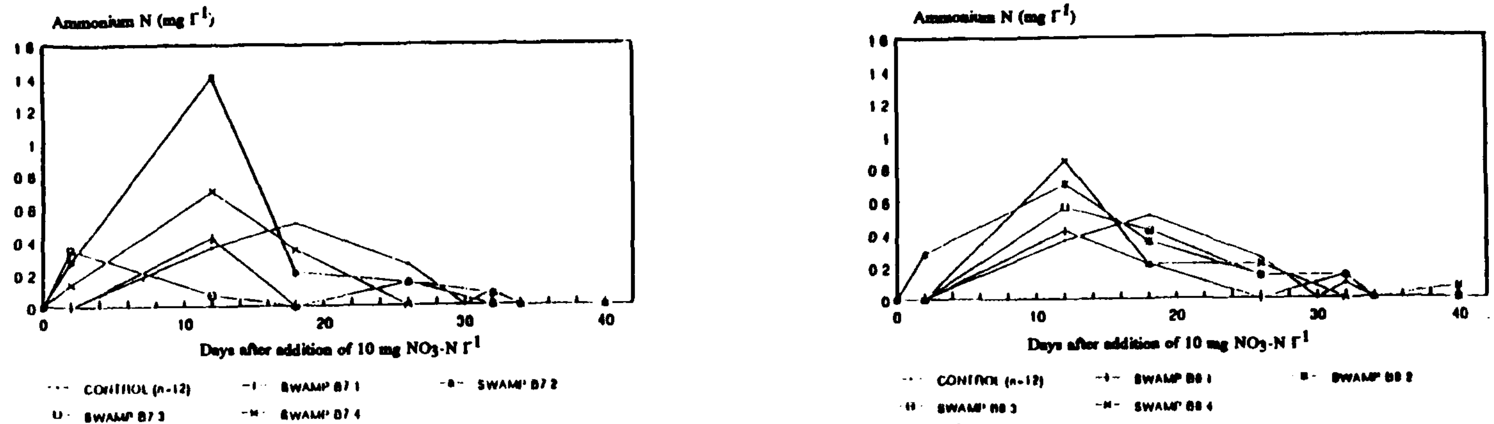
Nitrification

The rate of measured changes in the molar mass of ^{15}N atoms (Figure 4) in water has been used to predict rates of change in the molar masses of unlabeled atoms. Equal rates of change in the density of both isotopes have been assumed. Additions of ^{14}N, predicted according to rates of disappearance of ^{15}N, are then compared with actual measured changes in total nitrate concentration in water. This gives the results shown in Table 4, which indicates the size of the contributions of nitrification to the total nitrate pool. Amounts of nitrogen added by nitrification of organic material were usually less than



a. Sites B4, B7, and B8

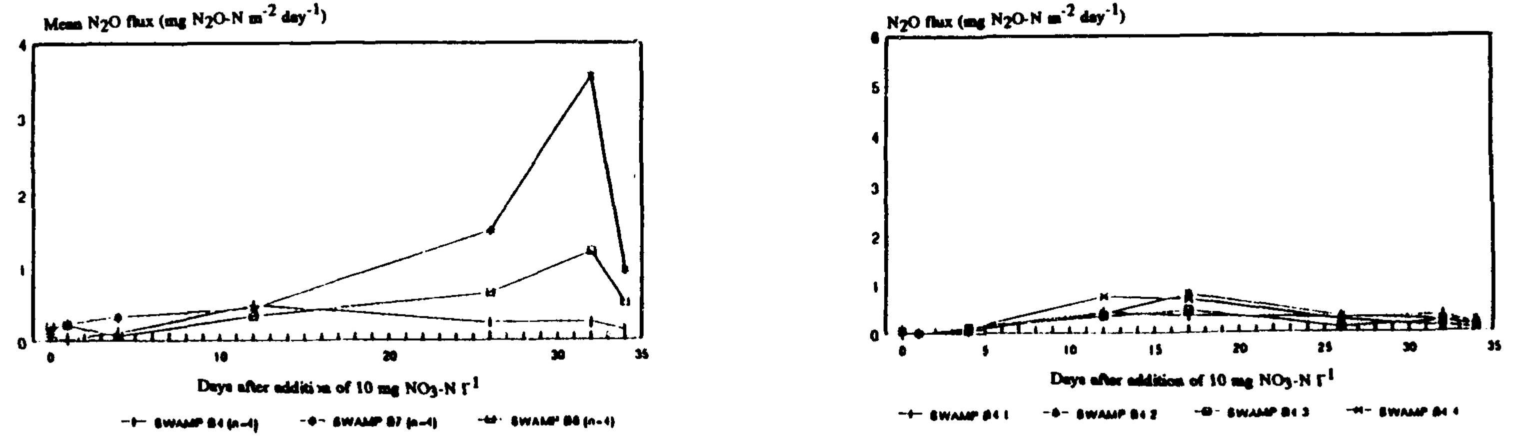
b. Site B4



c. Site B7

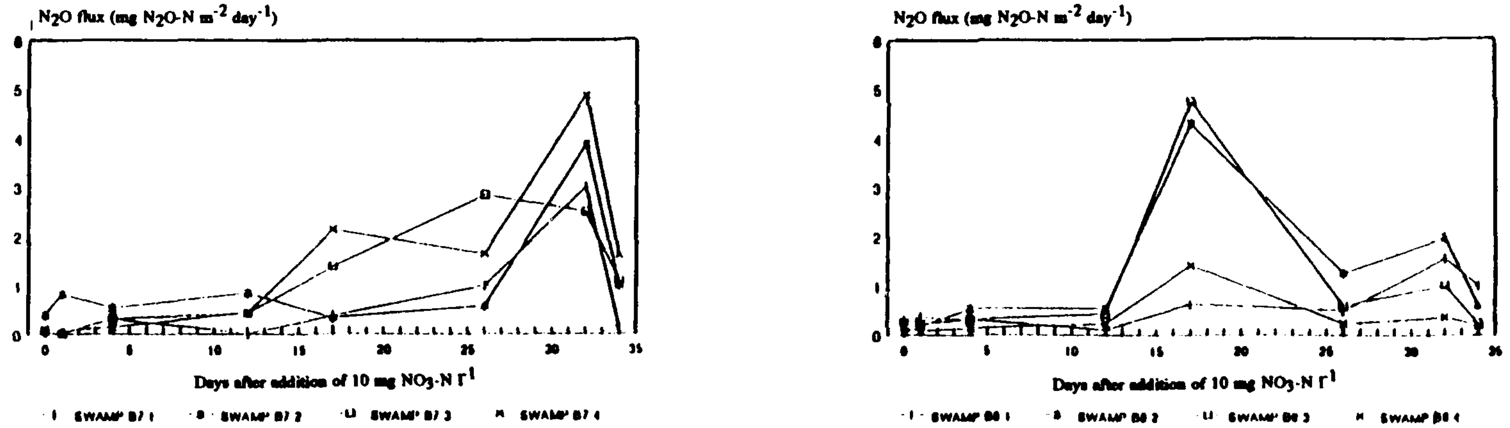
d. Site B8

Figure 5. Changes in mean (n = 4) concentration of ammonium-N in water overlying sediment from Sites B4, B7, and B8 and separated values for each of the sites



a. Sites 4, 7, and 8

b. Site 4



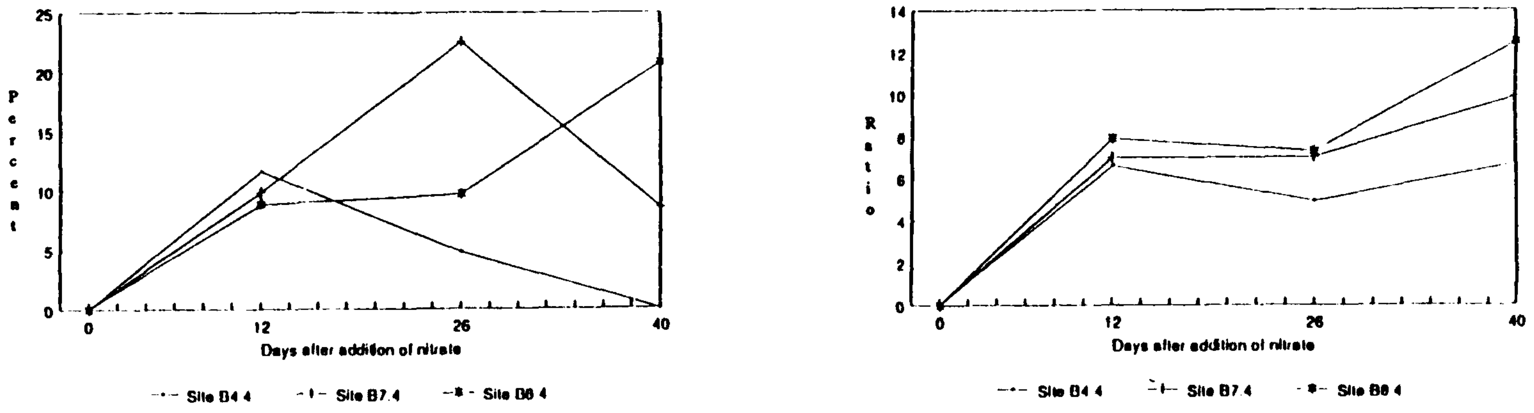
c. Site 7

d. Site 8

Figure 6. Mean (n = 4) fluxes of N_2O from water to the atmosphere from sediment-water columns

10 percent of the amounts of nitrate-N that were lost from water. The pattern of change in the contribution from nitrification to total amounts of nitrate in water (Figure 7) suggests that maximum rates of nitrification occurred before or on Day 12 for Site B4, around Day 26 for Site B7, and latest for Site B8.

A correlation did not occur between initial organic content of sediment and amounts of nitrate-N supplied from nitrification over the first 26 days. After Day 26, contributions of nitrate were greater from sediments with the highest organic content ($r = 0.9821$, $p < 0.02$). The ratio of nitrate supplied through nitrification to nitrate lost from water correlated directly with organic content during each of the time intervals (Days 0-12 and Days 12-26, $p < 0.05$; Days 26-40, $p < 0.001$).



a. Percent of total NO_3

b. Ratio of nitrification to NO_3 reduction

Figure 7. Contributions of nitrification to nitrate concentrations in water

4 Discussion

Nitrate concentrations of 5 to 10 mg NO_3-N L^{-1} in surface or groundwaters that have received drainage from cultivated land are not unusual (Smith, Alexander, and Wolman 1987; Turner and Rabalais 1991; Pionke and Urban 1984). Literature on models that predict extent of nutrient export are reviewed by Frink (1991). Inputs predicted from the extent and intensity of agricultural practice in such models are not usually sufficient to explain observed amounts of nitrogen in water. This is because of complications brought about by the biological nitrogen cycle. In already eutrophic lakes, and presumably in swamps too, nitrogen fixation may, for example, account for 6 to 82 percent of the nitrogen inputs (Howarth et al. 1988). Release of stored nitrogen from eutrophic sediments may also contribute substantial amounts of nitrate to water via nitrification (DeLaune and Smith 1987). At the same time, uptake of nitrogen by higher plants and N-losses to sediment as plant litter may provide temporary removal of nitrate from the water column. The contribution of soluble carbon from litter decomposition, which then becomes available to nitrogen-transforming bacteria, is thus of key importance in influencing the rate at which nitrate is both formed and lost. This is in evidence from the results of this study of swamp sediments.

Nitrate Reduction in the Floodwater

Rates of nitrate-N loss from water above the sediments studied here were 7.92 mg (Site 4), 11.32 mg (Site 7), and 9.62 mg (Site 8) m^{-2} day^{-1}. These rates fall within ranges reported for oligotrophic to mesotrophic lakes by Seitzinger (1988) from reviewed literature on denitrification. Rates given in this review for sediment from the Delaware River were between 28 and 58 mg N m^{-2} day^{-1} (calculated from the authors' figures) and for Lake Okeechobee sediment, 0.34 to 4.20 mg N m^{-2} day^{-1}. Patrick et al. (1976) found that for sediment sampled from freshwater swamp, removal of 100 mg NO_3 L^{-1} in overlying floodwater occurred after only 4 days. Rates of nitrate loss were thus low for sediments sampled from swamp environments where redox conditions are likely to favor nitrate loss. Results thus indicate wide variation in the potential of swamp sediments to process nitrate in floodwater.

On Day 30, about half of the nitrate added was still detected in the water column. Water temperatures of between 15 and 17 °C at the sediment surface were not likely to have limited rate of nitrate reduction (Seitzinger, Nixon, and Pilson 1984). Oxygen concentrations at the sediment surface were 0.2 mg L^{-1} in more than half of the cores and less than 0.3 mg L^{-1} in the others. These concentrations were at or below the upper limit for denitrification reported by Ronner and Sorensson (1985) and provided sufficiently oxidized conditions for nitrification to occur (Kemp et al. 1990). Redox potentials across a platinum wire electrode standardized with Quinhydrone were +200 mV at the water surface and -110 mV 1 cm below the sediment surface on Day 30. Redox measurements were not repeated, though together with reliable measures of oxygen concentration, were taken as sufficient evidence to suggest that redox conditions alone were not setting the rates of nitrate disappearance.

Carbon Limitation

Addition of glucose to half of the replicates more than doubled the rate of nitrate loss from all of the three sites and increased it by a factor of three in control bottles. This provides evidence that rates of nitrate disappearance were limited by availability of soluble carbon. These data therefore indicate that the low potential of these swamp sediments for nitrate removal is related to availability of an energy source for denitrifying bacteria. The organic content of surface sediment was low at about 6 percent from swamp Site B4, intermediate at 23 percent in Site B7, though high and the most typical of peaty wetlands in Site B8. Glucose amendments were based on the results of Moers (1990), who measured 36.4 mg glucose per gram dry weight in the top 10 cm of cypress peat. Assuming that the top 5 cm of the study sediments contained about 30 mg glucose per gram dry weight, approximately 7.5 g of glucose, to give exact concentrations of 1.04 mg L^{-1}, was added to each core.

Since river water that was not in contact with sediment lost only around 1.70 mg m^{-2} day^{-1}, the influence of swamp sediment increased rates of nitrate loss in the water column, on average, sixfold. River water contained conspicuous amounts of filamentous algae at the start of the experiment. Because incubations were carried out in darkness, any nitrate loss because of denitrification in river water was likely to have depleted available carbon within a very short time. Cores, which were also kept in darkness, had much larger initial stores of organic matter. Their rate of net primary production over the time scale of the experiment would have been negligible compared with eutrophic river water. Rates of nitrate loss from within the water column calculated from control bottles are thus more conservative than estimates for sediment-water columns. As expected, the difference in rates between cores and water became less after the addition of glucose.

Contribution of Nitrate from Nitrification

Sediments may supply substantial amounts of ammonium for nitrification. Because they provide a matrix where both oxidizing and reducing conditions can coexist, their ecological role is as both source and sink for nitrate. In these conditions, the amount by which nitrate is lost must be balanced against the amount of nitrate added to water by nitrification before predictions of final nitrate concentrations can be made.

Calculations of the contribution of nitrate from nitrification made here rely on the assumption that the rate of supply of ^{14}N atoms from nitrification is equal to the rate of change in the mass of ^{15}N atoms in water. This assumes that ^{15}N atoms and ^{14}N atoms are equally likely to be removed by denitrification, and that there is therefore no selectivity for ^{14}N. Predicted additions of ^{14}N over each time interval are then compared with the actual measured changes in total nitrate concentration in different sample aliquots. Their fractional contribution to the totals at each time may then be derived. If the assumption that ^{14}N is not removed selectively holds, then our equations provide an accurate estimate of nitrification, which does not involve changes in the isotopic ratio of ammonium. This is an advantage where sediments yield only small amounts of ammonium, which may then be large compared with amounts formed by dissimilatory reduction of nitrate to ammonium. Koike and Hattori (1978) were able to ignore dissimilation because natural amounts of ammonium in sediment were relatively large.

In this study, nitrification was a relatively unimportant influence on nitrate concentration in floodwater. Nitrification contributed only around 10 percent to the nitrate presumed lost by denitrification. This is lower than figures for shallow and eutrophic drainage basins in Louisiana where the production of nitrate by nitrification was around 60 percent of the nitrate reduction rates (DeLaune and Lindau 1989). The relative importance of nitrification in the sediment-water columns studied here was greatest for the most organic sites. DeLaune and Smith (1987) measured contributions of 70 percent in Lake Verret. This lake receives high loadings of nitrogen from agricultural runoff. These observations together suggest that in shallow wetlands and lakes the importance of nitrification increases with increasing eutrophication and may eventually equal losses because of denitrification.

Both nitrification and denitrification can be limited by availability of soluble carbon. Results for Cache basin sediments show that the relative importance of nitrification compared with nitrate reduction (the ratio of nitrate addition to nitrate reduction) was least in the system with the smallest amount of plant debris and consistently greatest in the most organic site. Absolute contributions of nitrate through nitrification followed a pattern of decline after Day 12 for the least organic site, decline after Day 26 for the intermediate site, and continuing increase for the most organic site. Because nitrate reduction was limited by carbon in these systems, results suggest that rate of litter decomposition is a strong influence on the contribution of nitrification to the nitrogen cycle. Rates of supply of ammonium must be related to the proportions of refractory tissues in organic matter. These proportions are likely to be higher in forested swamp than in grass-dominated wetlands. Limits on nitrogen cycling set by carbon are an important feature of terrestrial soils. Drury, Kenney, and Findlay (1991), for example, examined relationships between denitrification (measured by acetylene inhibition) and a variety of properties of 13 different soils. They found that nitrate concentrations were less strongly correlated with denitrification rates than the organic-carbon content of the soils. They also added dextrose to soil and were able to conclude that it was the availability of soluble carbon, and none of the other soil properties, that limited denitrification.

Direct Measurement of Denitrification

That reduction of nitrate concentrations in water overlying sediment occurred during this experiment does not confirm that the major pathway of loss was denitrification. Conclusive evidence can only be gained where accumulation of free nitrogen gas is measured and an account can be given of other losses of nitrogen from the enclosed system. Head space gas was analyzed for its $^{15}N_2$ enrichment in this study, using a Finnagen Mat dual inlet mass spectrometer, to give a direct measure of denitrification. Results were not presented earlier because recoveries of ^{15}N in the head space were small. Amounts of $^{15}N_2$ measured in the head space in this experiment were around four times higher (20-percent rather than 5-percent recovery, $p < 0.02$) in the replicates that were treated with glucose. These data, together with the lack of any correlation between concentration of either N_2 or N_2O concentration and the length of time for which cores were presumed gastight, suggest that leakage of gas out of the system was not a significant problem. Although it is clear that nitrate reduction occurred in the water column, the precise fate of this nitrogen in unknown.

A proportion of gaseous products of denitrification could remain trapped within the sediment matrix. Reddy, Patrick, and Lindau (1989) in their study of nitrification-denitrification at the plant root-sediment interface found that 88 to 94 percent of added 50 atom percent excess KNO_3 added to sediment-water columns could not be accounted for at the end of their experiments. In columns that contained rice plants, 24 percent of the denitrified gases were recovered as N_2 and 76 percent were unaccounted for. The authors supposed that denitrified gases stayed trapped in sediment from evidence by Lindau et al. (1988), who showed that 40 percent of the $K\,^{15}NO_3$ applied to soil as fertilizer remained trapped as N_2 in soil 33 days after fertilization. This may account for at least some, but doubtfully all, of the shortfalls observed here. In sealed cores where light is excluded or maintained at low intensity, there would be little or no phototrophic fixation of trapped N_2 by blue-green algae, which could then lead to its accumulation. Nitrogen-fixing blue-green algae are an important component of benthic sediments, especially in conditions of nutrient enrichment. Low light intensity may therefore interrupt the cycling of nitrogen between denitrification and fixation. Addition of more highly enriched isotope, incubations at ambient light intensity, and sufficient replicates to allow destructive sampling of sediment so that any trapped gases can be removed might lead to improved recovery rates.

Work by Dalva and Moore (1991) implies that natural rates of nitrate disappearance may be underestimated by sediment-water columns because these are isolated from inputs of labile carbon in canopy leachate. They found that after passage through tree canopies, the amount of dissolved organic carbon (DOC) in precipitation increased from 2.0 mg L^{-1} to 9.1-14.6 mg L^{-1} as throughfall, and to 23.1-30.1 mg L^{-1} as stemflow. The increases appeared greater under coniferous than under deciduous trees. It is therefore plausible that DOC in throughfall contributes an important part of the soluble carbon in surface water. Dalva and Moore also suggest that mineral sediments may provide a sink for DOC through adsorption onto iron and aluminum. Together, these factors help to explain both why carbon-limited nitrogen transformation and why this may also happen in the natural environment.

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Table 1
Mean Rates and Amounts of Nitrate Disappearance over 40 Days from Floodwater Overlying Sediment from Bottomland Hardwood Forest Swamp (n = 4 until Day 31, then n = 2)

Parameter	$[NO_3\text{-}N]$(mg L^{-1}) =	R	P	NO_3-N lost g m^{-2}
Site B4	9.60 - 0.14 days	-0.9689	< 0.001	0.30
Site B7	9.50 - 0.20 days	-0.9920	< 0.001	0.46
Site B8	9.57 - 0.17 days	-0.9701	< 0.001	0.39
River Water	8.98 - 0.03 days	-0.7448	not sampled	0.00

Table 2
Rates of Nitrate Disappearance (y) over 7 Days from Floodwater Overlying Sediment from Bottomland Hardwood Forest Swamp with and without the Addition of 1.04 g Glucose L^{-1}

With Glucose	Without Glucose
Site R	Site R
B4(Rep 1) y = 49.51 - 1.45 days - 0.9813	B4(Rep 1) y = 12.25 - 0.22 days - 0.9851
B4(Rep 2) y = 71.81 - 2.10 days - 0.9578	B4(Rep 2) y = 11.38 - 0.18 days - 0.9810
B7(Rep 1) y = 53.49 - 1.57 days - 0.9693	B7(Rep 1) y = 6.70 - 0.12 days - 0.9477
B7(Rep 2) y = 39.75 - 1.17 days - 0.9804	B7(Rep 2) y = 7.12 - 0.14 days - 0.9778
B8(Rep 1) y = 55.82 - 1.63 days - 0.9217	B8(Rep 1) y = 9.13 - 0.17 days - 0.9585
B8(Rep 2) y = 48.82 - 1.42 days - 0.9449	B8(Rep 2) y = 11.77 - 0.24 days - 0.9835
Swamp Sites	
Mean ± s.d. rate - 1.56 ± 0.31 mg NO_3 day^{-1} ($p < 0.01$)	-0.18 ± 0.04 mg NO_3 day^{-1} ($p < 0.01$)
River Water	
Mean ± s.d. rate - 1.15 ± 0.14 mg NO_3 day^{-1} ($p < 0.01$)	0.01 ± 0.05 mg NO_3 day^{-1} (n.s.)

Table 3

Linear Regression Equations Describing mean (n = 4) Rates of Evolution of N_2O (mg N_2O-N m^{-2} day^{-1}) at Intervals over 17 Days and Total N Lost as N_2O after 34 Days from Flooding Bottomland Hardwood Forest Swamp with Water Containing 9.27 mg NO_3-N L^{-1}

Location	Rate of N_2O Evolution =	R	P	N_2O-N lost mg^{-2}
Site B4	-4.53 + 9.45 days	0.9879	< 0.001	0.17
Site B7	-2.78 + 12.00 days	0.9174	< 0.01	1.99
Site B8	-94.62 + 35.60 days	0.7797	< 0.05	0.76

Table 4

Amounts of Nitrate-N (mg NO_3-N L^{-1}) Lost Through Denitrification over Each Time Interval and Amounts of Nitrate Gained Through Nitrification over the Sample Period for Replicate (4 from each site)

Location	Days 0-12		Days 12-26		Days 26-40	
	NO_3 lost	NO_3 gain	NO_3 lost	NO_3 gain	NO_3 lost	NO_3 gain
Site B4	3.79	0.25	1.83	0.09	0.27	0.02
Site B7	3.71	0.26	3.68	0.26	0.83	0.08
Site B8	3.04	0.24	2.59	0.19	1.20	0.15

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13. ABSTRACT *(Maximum 200 words)*

To give initial nitrate-N concentrations of about 9 mg L^{-1}, ^{15}N labeled nitrate was added to sediment-water columns containing wetland sediment and river water. Over 40 days, nitrate-N in floodwater decreased by between 82 and 59 percent, which gave estimates of N export from the water column of 11.5 mg N m^{-2} day $^{-1}$ and 7.5 mg N m^{-2} day^{-1}. These values are low compared with wetland systems elsewhere and correlated directly with the organic content of surface sediment, which included the forest litter. Added glucose at least doubled rates of nitrate loss, which were then presumed to have been carbon limited. Nitrification was estimated using changes in atom percent $^{15}NO_3$ alone and not changes in atom percent NH_4, which is a more usual method. Contributions of NO_3 to water from nitrification were small related to initial organic contents and were estimated at between 5 and 12 percent of the amounts by which nitrate reduced. Rates of nitrogen transformation in these forested swamps are likely to be set by rate of supply of soluble carbon from tree litter and perhaps canopy leachate, rather than by nitrate concentration in inflowing floodwater.

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