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DENITRIFICATION IN BOTTOMLAND HARDWOOD WETLAND SOILS OF THE CACHE RIVER

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Abstract: Denitrification rates were quantified in bottomland hardwood wetland soils of the Cache River. N^{15} labeled nitrate was added to columns containing wetland soils and river water. Over 40 days, nitrate-N in floodwater (approximately 9 mg N L^{-1}) decreased by between 82% and 59%, which gave estimates of N export from the water column of between 11.5 mg N m^{-2} day^{-1} and 7.5 mg N m^{-2} day^{-1}. These values correlated directly with organic content of surface sediment, which included forest litter. Added glucose doubled rates of nitrate loss in these soils, which indicated that the process was carbon limited. Nitrification occurring simultaneously with denitrification was determined using isotopic dilution techniques. Contributions of nitrate to water from nitrification were estimated at between 5% and 12% of the total nitrate reduced. Rates of nitrogen transformation in these forest soils were likely limited by available soil carbon from tree litter and perhaps canopy leachate, rather than by nitrate concentration in inflowing floodwater.

Key Words: bottomland hardwoods, Cache River, denitrification, nitrogen, nitrification, soil carbon

INTRODUCTION

Denitrification of floodwater in swamps and shallow lakes has significant consequences for both wetland ecology and for management of water quality. Each winter and spring, the Cache River in northeastern Arkansas 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 south-central United States. Denitrification, which is the biological reduction of nitrate-N to nitrous oxide and nitrogen gas, can lead to losses of nitrogen from wetland sediments and oxygen-depleted water. Rates of nitrogen loss vary greatly between different ecosystems, although they 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 freshwater. 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 (Knowles 1982, Fillery 1983, Beauchamp et al. 1989). 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.

The ecological response of bottomland hardwood wetlands and cypress swamp to nutrient enrichment and, in the shorter term, the capacity to denitrify floodwater are relatively unstudied. Decomposition of organic matter produced within natural forested swamps can be slow, with as much as 40% 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 nitrogen inputs in drainage from agriculture. Since nutrient enrichment of relatively closed aquatic ecosystems leads to the inevitable accumulation of deep organic sediments, the potential exists in these sediments 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) have measured the contribution of internal ammonium pools in benthic sediment and found that the rate of conversion of ammonium to nitrate was around 70% of the rate of loss of nitrate through denitrification.

Water chemistry studies performed prior to this study showed a significant loss of nitrate between a sampling point on the Cache River upstream from the bottomland hardwood wetland and a sampling point downstream (G. Godshalk, pers. comm.). The objective of the study was to examine sediment-related nitrogen processing in different portions of a floodplain forest. This was accomplished using a laboratory-based experiment to quantify nitrogen changes in sediment cores and in the overlying water columns as related to carbon availability.

MATERIALS AND METHODS

Site Description

Sediment cores were obtained from the floodplain of Cache River, located in the lower Mississippi River alluvial valley in eastern Arkansas (Figure 1). Site B4 was located about 120 m from the bank of the Cache River, in a backwater area dominated by water tupelo (*Nyssa aquatica* L.), water elm (*Planera aquatica* Gmel.), and (*Taxodium distichum* (L.) Rich). The soil was a Typic Ochraqualf, which was heavily gleyed within 25 cm of the surface. Using the hydrologic model developed for the project (Walton et al. 1996), it was estimated that the site was flooded an average of 271 days a year during the study period. Site B7 was located 540 m away from the Cache River, on a ridge dominated by American elm (*Ulmus americana* L.), sweetgum (*Liquidambar styraciflua* L.), and persimmon (*Diospyros virginiana* L.). The soil was a Albic Glossic Natraqualf, and the site was flooded an average of 84 days a year. Site B8 was 660 m away from the river in the "second bottom." While some American elm was still present, the vegetation moved to more water-tolerant species than B7, and was dominated by overcup oak (*Quercus lyrata* Walt.), swamp privet (*Forestiera acuminata* Poir.), red maple (*Acer rubrum* L.), and a few cypress. The soil at this site was a Fluventic Haplaquept. Although the elevation of the site indicates that the area would be flooded an average of 98 days per year, the site is actually a perched area, and water ponds on the site much longer, perhaps as much as 150 days a year.

Addition of Nitrate to Sediment-Water Columns. Four 15-cm-diameter by 25-cm-depth cores of wetland sediment with intact plant litter were collected from each site (B4, B7, and B8) using 100-cm-length PVC tubing. At the same time, approximately 200 liters of river water were collected. The materials were sampled after drawdown in October 1991. Sediment cores were stored at field capacity at 2 °C until January 1992, when they were sub-sampled 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 although 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 liters. Floodwater volumes were adjusted to give the same head space volume of 1.325 liters in each core.

Each of the twelve cores was allocated at random to one of four large containers that were filled with tap water intended to buffer against changes in external temperature. Three gastight 0.5-liter bottles with removable screw-tops were floated in each of these large outer containers. These control bottles containing unfiltered river water were used to monitor changes in nitrogen in the river water not associated with the sediment.

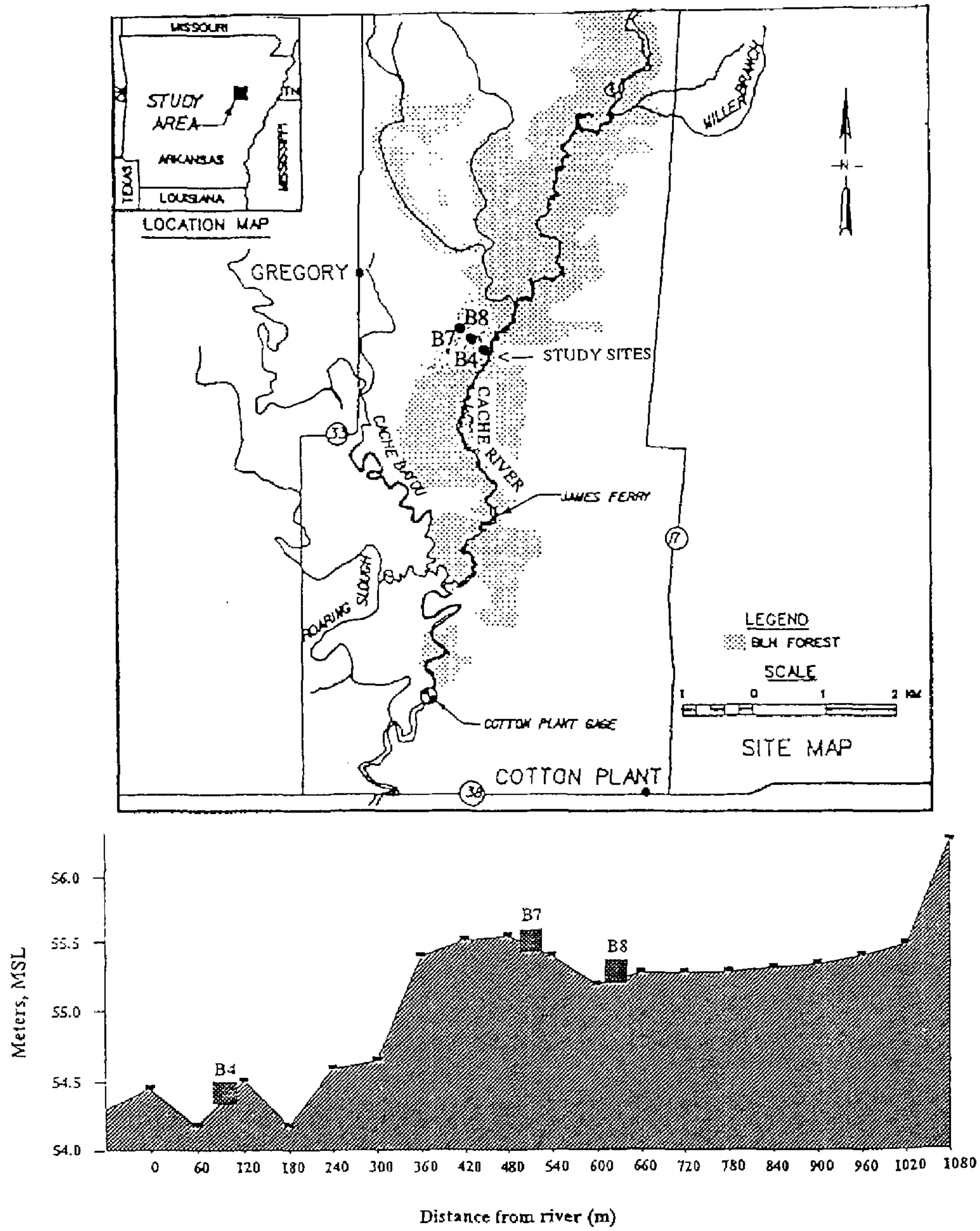


Figure 1. Location map of study area and cross-section of the Cache River floodplain depicting the elevation of the sample site and their distance from the river.

After 10 days equilibration, 3 cores from each site received aliquots of 61.5 atom % N^{15} 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 % N^{15} in solution were measured on water sampled from the twelve 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 (1 for each site) were incubated at ambient laboratory temperature, which was maintained at around 22 °C.

Temperature and oxygen concentrations at 10-cm-depth intervals in the sediment-water columns were measured using an oxygen probe. Measurements were made at the beginning, during the middle, and towards the end of the experiment. Samples of water and gas were taken at increasing time intervals for 12 hours and then at varying intervals over 40 days. Larger volumes of water (ranging from 0.20 liters to 2 liters) for determination of isotopic ratio were sampled at the same times as 10-ml samples, although less frequently and only on days 1, 12, 26, and 40. The 2-liter samples were required only at the end of the experiment (day 40) when the cores contained low inorganic nitrogen levels.

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. The glucose addition was to assess the importance of carbon limitation on nitrogen dynamics.

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% for nitrate-N and to ± 0.03% for ammonium-N. Duplicate distillates of larger sample volumes were combined, acidified, and evaporated 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 residual gas analyzer, which is sensitive to 0.001 atom % N-15.

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

and the constants A and B are given by

$$A = (f)f^d/(f^d)$$

$$B = (f)/(f^d)$$

where

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

These equations differ from the equations given by Koike and Hattori (1978) that use changes in the isotopic ratio of ammonium as well as the depletion of N^{15} labelled 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 N^{15} labeled nitrate in water. An essential condition for application of these equations is that measurements of total nitrate and of N^{15} labelled nitrate 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% or better. Data analysis were conducted using Statistical Analysis System (SAS). Linear regression and ANOVA procedures were used to determine rates of NO_3^- loss and the presence significant correlations.

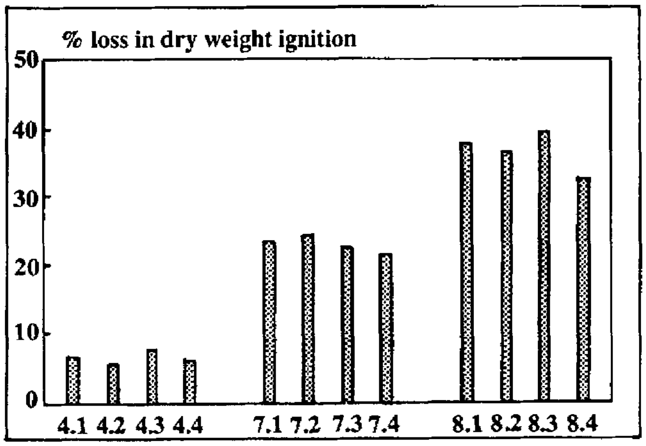


Figure 2. Initial organic content of each of the 12 cores from the 3 sites.

RESULTS

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

Average 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. Average oxygen concentrations at the top of the water column in core B4 were always higher ($p<0.05$) than for the other two sites, although 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. Redox potentials across a platinum wire electrode standardized with Quinhydrone were +200 mV at the waters' surface and −110 mV 1 cm below the sediment surface on day 30. 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% and differed between the three sites ($p<0.001$). Values ranked from 6.9±1.0% in site B4, where the sediment was a fine sandy loam, through 23.2±1.3% in site B7, to 36.9±3.0% 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 82.2±3.7% of its initial nitrate and site B4, where nitrate disappeared

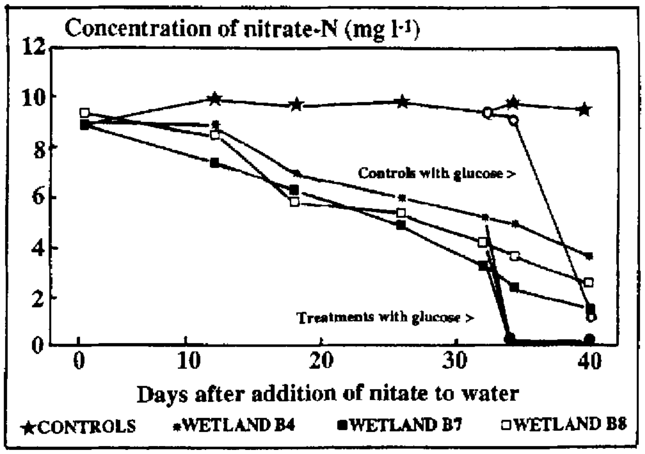


Figure 3. Changes in mean concentration of nitrate in water flooding bottomland hardwood sediments and in control bottles that contain river water and no sediment (pairs that received glucose are shown seperately after Day 30).

most slowly, only 59.2±2.8% (Figure 3). Table 1 describes rates of nitrate disappearance, which 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 16.4±12.9% of its initial nitrate-N and was the only site to differ ($p<0.02$) from controls at this time. Controls contained 8.9±0.5 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.

Approximately half (site B4: 45.1±10.6%, site B7: 65.4±6.4%, site B8:58.3±5.4%) of the floodwaters initial NO$_3$-N was lost by day 30. The nitrate loss from site B4 at this time was less than from sites B7 and B8 (site B7: $p<0.001$, site B8: $p<0.05$), and there was no difference in rate of nitrate loss between sites B7 and B8.

The rate loss of NO$_3$-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 three 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 NO$_3$-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 (ca 1.5 mg NO$_3$-N l^{-1} day^{-1}) than in control bottles (ca 1.0 mg NO$_3$-N l^{-1} day^{-1}).

On day 12, there were no correlations between dissolved oxygen at any depth and nitrate concentration in the twelve cores. By day 28, direct although usually weak correlations emerged between oxygen (at the sediment surface, $r=0.47$, $p<0.06$) and nitrate concentrations.

Changes in the mass of N^{15} atoms in the dissolved inorganic nitrogen fraction of the total volume of water in each core are shown in Figures 4(a) to (f). Water from replicates (4 from each site) was analyzed for nitrate (including nitrite) and ammonium separately. In all sites, there was a steady decrease in the amount of N^{15} labeled nitrogen in water (in B7, by −1.14 mg N^{15} per core per day, $p<0.05$; and in B8, −1.19 mg N^{15} per core per day, $p<0.02$), with slowest disappearance from site B4 (by −0.95 mg N^{15} per core per day, $p<0.01$). Glucose treatments lost all their N^{15} in water between day 32 and day 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.

The low concentration of ammonium measured in the water over time became enriched with N^{15} to approximately 12 atom percent. Isotope added to sites B7 and B8 was diluted over time to approximately 50% of initial concentrations (Figure 4(a) to (f)). Change in isotope ratios were initially slow. Atom percent of N^{15} in the water from these cores decreased significantly ($p<0.05$) and at a faster rate after 28 days.

Concentrations of ammonium in sites B7 and B8 were sufficient, although 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 N^{15} in ammonium at the three sites was 13.2±1.2% on day 12 and reached a mean enrichment of 21.9±9.5% 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 N^{15}.

Table 1. Linear regression equations describing 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 $n = 2$).

Location	[NO$_3$-N](mgl^{-1})	F-value	r	P	NO$_3$-N Lost g m^{-2}
Site B4	9.60–0.14 days	14.96	−0.9689	<0.001	0.30
Site B7	9.50–0.20 days	45.81	−0.9920	<0.001	0.46
Site B8	9.57–0.17 days	27.37	−0.9701	<0.001	0.39
RIVER WATER:	8.98–0.03 days	n.s.	−0.7448	n.s.	0.00

n.s.—not significant.

Table 2. Rates of nitrate disappearance (y) over day 31–40 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
4 (Rep 1)	y = 49.51–1.45 days–0.9813	4 (Rep 1)	y = 12.25–0.22 days–0.9851
4 (Rep 2)	y = 71.81–2.10 days–0.9578	4 (Rep 2)	y = 11.38–0.18 days–0.9810
7 (Rep 1)	y = 53.49–1.57 days–0.9693	7 (Rep 1)	y = 6.70–0.12 days–0.9477
7 (Rep 2)	y = 39.75–1.17 days–0.9804	7 (Rep 2)	y = 7.12–0.14 days–0.9778
8 (Rep 1)	y = 55.82–1.63 days–0.9217	8 (Rep 1)	y = 9.13–0.17 days–0.9585
8 (Rep 2)	y = 48.82–1.42 days–0.9449	8 (Rep 2)	y = 11.77–0.24 days–0.9835
WETLAND 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.)	

Changes in Ammonium Concentration

Ammonium-nitrogen was undetectable in river water before the addition of nitrate to cores on day 0. After one day, three of the cores from site B7 had a 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,b,c,d). Ammonium seemed to accumulate in the water overlying sediments from sites B7 and B8 until around day 12, in control bottles containing river water not until eight days later, and not at all in water that flooded site B4. Although there were significant differences between sites B4 ($p<0.05$) and B8 ($p<0.05$) and controls, there was no difference 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, although 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.

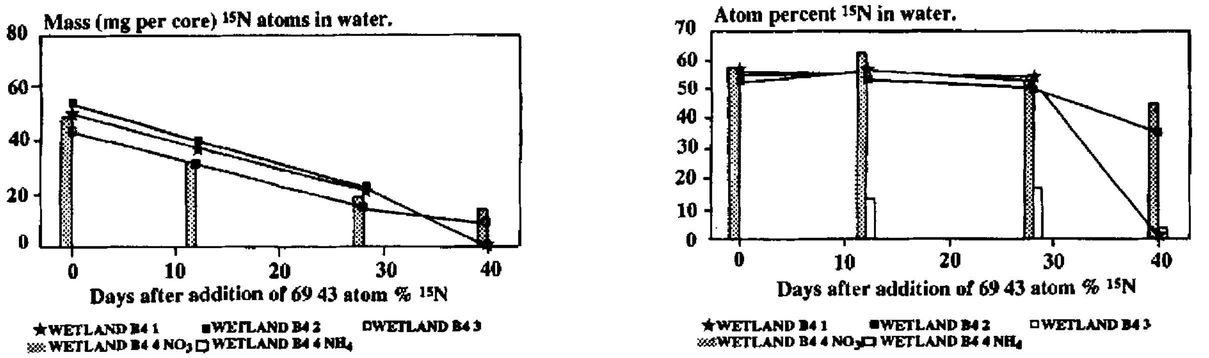
Production of Nitrous Oxide

Rates of N_2O production are expressed as nitrogen fluxes between water and air in units of mg N_2O-N evolved per m^2 sediment per day (Figure 6). As Table 3 demonstrates, the rates of N_2O fluxes were highest from site B8 and the lowest from site 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% of those from the other two sites. The relationship between change in nitrate concentration over all twelve 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% of the variation in nitrate concentration over the 40 days and with 37.9% over the first 17 days after flooding.

Nitrification

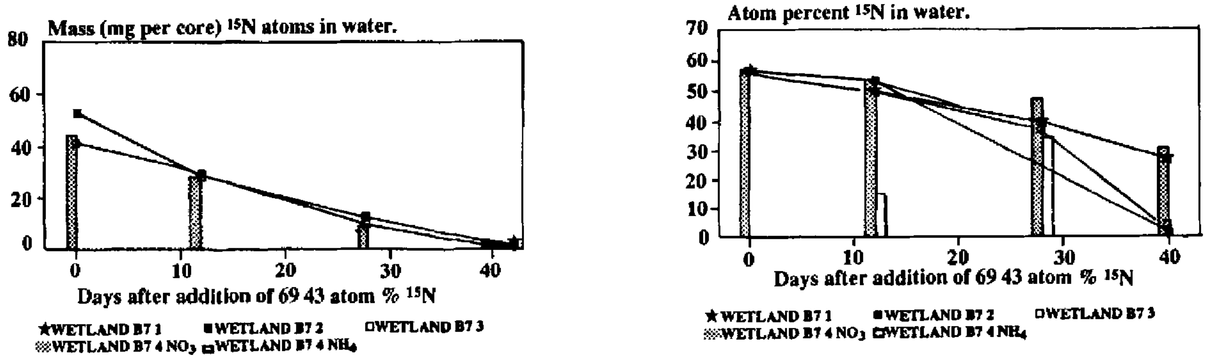
The rate of measured changes in the molar mass of N^{15} atoms (Figure 4) in water have been used to predict rates of change in the molar masses of unlabeled atoms. Equal rates of change in the density of both isotopes have been assumed. Additions of N^{15}, predicted according to rates of disappearance of N^{15}, are then compared with actual measured changes in total nitrate concentration in water. This gives the results shown in Table 4, which indicate 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 10% 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 4) 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.

There was no correlation 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



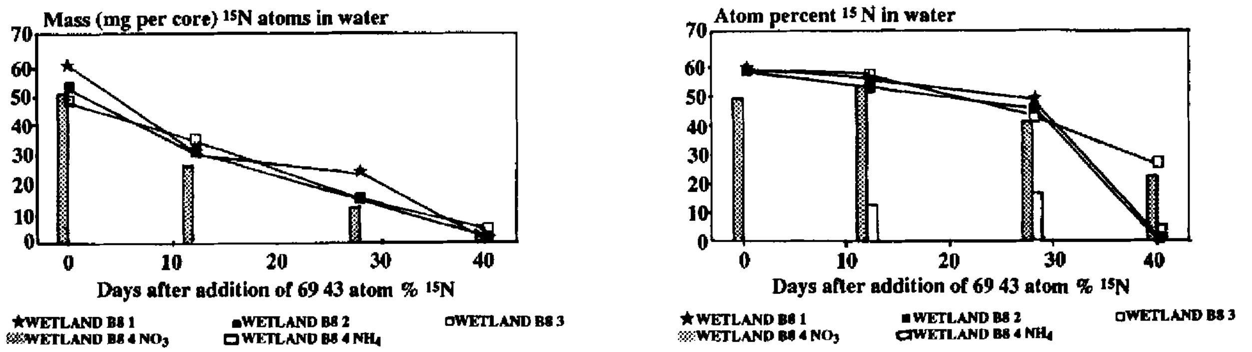
a. Change in total mass of ^{15}N atoms/mg per core in water column.

b. Change in percentage ^{15}N in 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.



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

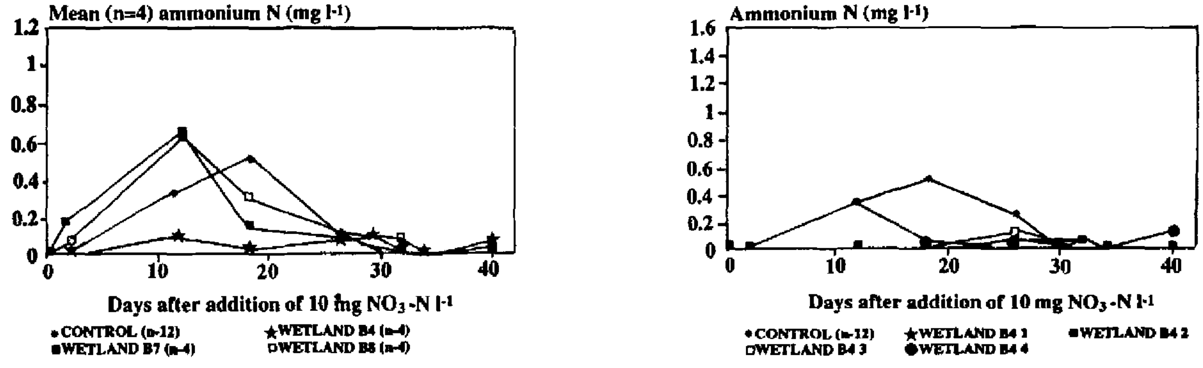
f. Change in percentage ^{15}N in water column over time

Figure 4. Changes in total mass of N^{15} atoms dissolved inorganic nitrogen in the water column of each core and changes in percentage of N^{15} atoms in water over time.

content during each of the time intervals (days 0–12 and days 12–26, $p<0.05$, and 40, $p<0.001$).

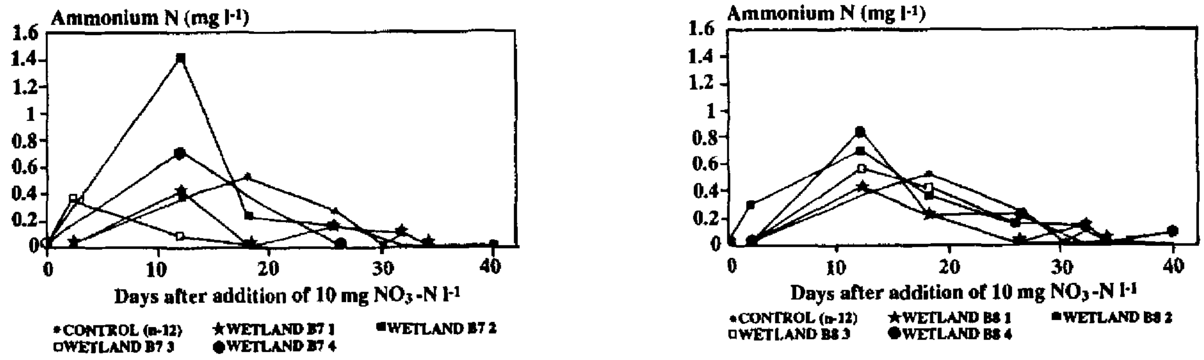
DISCUSSION

Nitrate concentrations of 5 to 10 mg NO_3-N l^{-1} in surface waters or ground waters that have received drainage from cultivated land are not unusual (Pionke and Jurban 1984, Smith et al. 1987, Turner and Rabalais 1991). 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% of the nitrogen inputs (Howarth et al. 1988). Release of stored nitrogen from eutrophic sediments may also contribute substantial



a. Sites B4, B7, and B8

b. Site B4



c. Site B7

d. Site B8

Figure 5. Changes in mean concentration of ammonium-N in water overlaying sediment from sites B4, B7, and B8 and seperated values for each of the sites.

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 forested wetlands.

Nitrate Reduction in Floodwater

Rates of nitrate-N loss from water above the sediments studied here were 7.92 mg (site B4), 11.32 mg (site B7), and 9.62 mg (site B8) 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 author's 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. Lindau et al. (1988) report relatively low nitrate reduction rates (2.7 to 5.2 mg N d^{-1}) for a lower Mississippi River valley deltaic plain swamp forest, which supports the nitrate reduction rates measured at the Cache River study sites.

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 et al. 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 also (Kemp et al. 1990). Redox measurements 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.

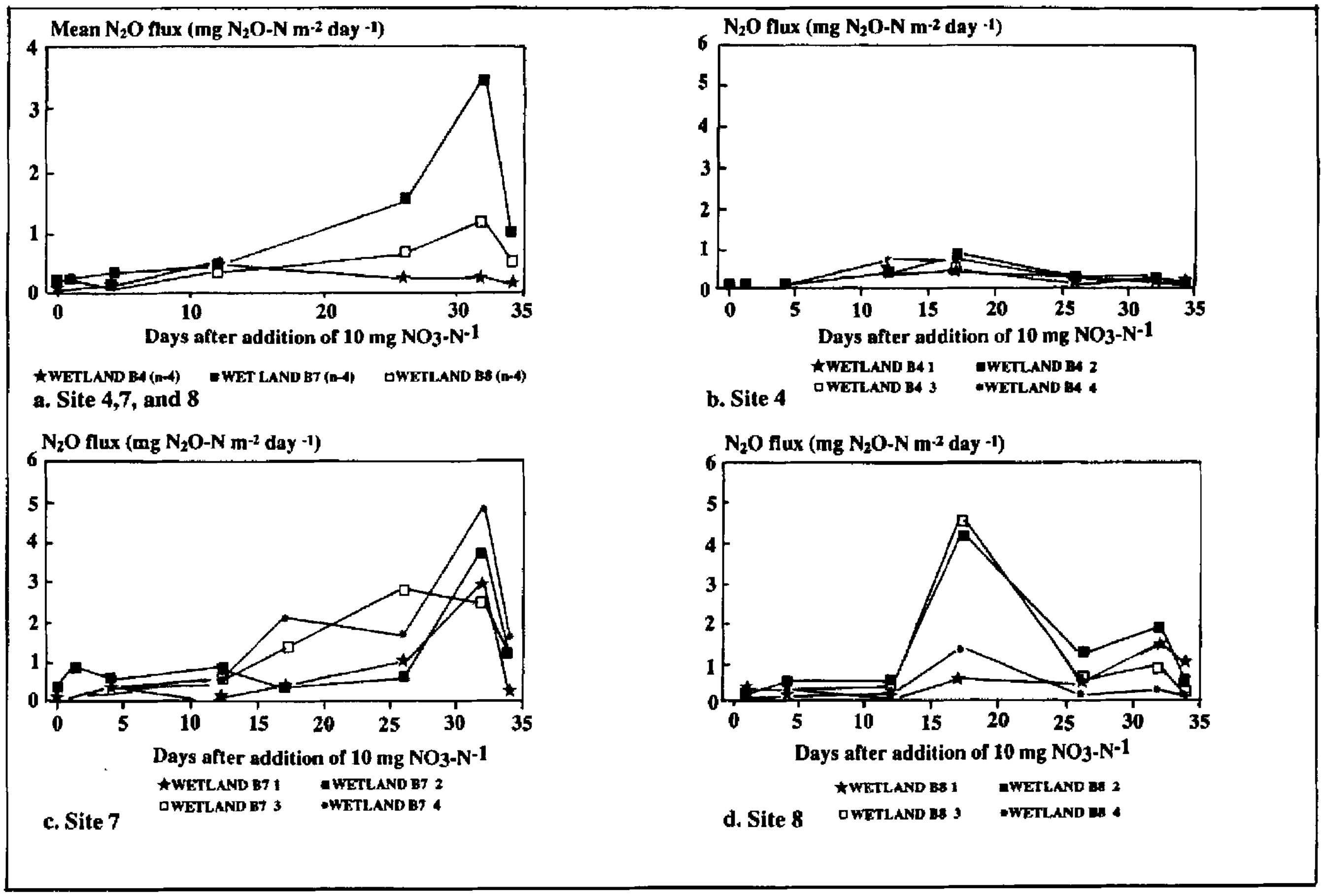


Figure 6. Mean fluxes of N_2O from water to the atmosphere from sediment-water columns.

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 ca 6% from swamp site B4, intermediate at 23% in site B7, and highest at site B8 at 36%. Although the rate of nitrate reduction was highest for site B7, the organic content of site B8 was highest. A possible explanation for this exists in that much of the leaf litter in the ponded area at site B8 is recalcitrant cypress needles, which may not be a good carbon source for the microbes as the more labile leaves present in the vicinity of site B7. 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.

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} (dF = 3).

Location	Rate of N_2O Evolution	F Value	R	P	N_2O Lost mg^{-2}
Site B4	−4.53 + 9.45 days	167.03	0.9879	<0.001	0.17
Site B7	−2.78 + 12.00 days	34.12	0.9174	<0.01	1.99
Site B8	−94.62 + 35.60 days	10.13	0.7797	<0.05	0.76

Table 4. Amounts of NO_3-N (mg NO_3-N l^{-1}) lost over each time interval and amounts of nitrate gained through nitrification over the sample period for replicate (4 from each site).

	Days 0–12		Days 12–26		Days 26–40	
Location	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

Since river water that was not in contact with sediment lost only about 1.7 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, there would have been little nitrogen uptake and/or growth of this algae. Any nitrate loss due to denitrification in river water would have depleted available carbon (resulting from the algae) within a very short time. The presence of algae would have little effect on the results. 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 to 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 co-exist, 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 N^{14} atoms from nitrification is equal to the rate of change in the mass of N^{15} atoms in water. This assumes that N^{15} atoms and N^{14} atoms are equally likely to be removed by denitrification and that there is, therefore, no selectivity for N^{14}. Predicted additions of N^{14} 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 N^{14} is not removed selectively holds, then our equations provide an accurate estimate of nitrification that 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 disassimilation because natural amounts of ammonium in sediment were relatively large.

In this study, nitrification did not significantly influence nitrate concentration in floodwater. Nitrification contributed only around 10% 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% 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% in bottom sediment for a coastal Louisiana lake receiving high loading 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 due to 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 has 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 wetlands than in grass-dominated wetlands. Limits on nitrogen cycling set by carbon are an important feature of terrestrial soils. Drury et al. (1991), for example, have examined relationships between denitrification (measured by acetylene inhibition) and a variety of properties of thirteen 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 other losses of nitrogen from the enclosed system can be accounted for. Head space gas was analyzed for its $^{15}N_2$ enrichment in this study, using a Finnigan Mat Delta E dual inlet mass spectrometer, to give a direct measure of denitrification. Results were not presented earlier because recoveries of N^{15} 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%, rather than 5% recovery, $p<0.02$) in the replicates which 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 et al. (1989) in their study of nitrification-denitrification at the plant root-sediment interface found that 88% to 94% of added 50 atom% 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% of the denitrified gases were recovered as N_2 and 76% were unaccounted for. The authors supposed that denitrified gases stayed trapped in sediment from evidence by Lindau et al. (1988), who showed that 40% 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 we doubt all, of the shortfalls observed here. In sealed cores where light is excluded or maintained at low intensity, there would be no or little 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 to 14.6 mg l^{-1} as throughfall and to 23.1 to 30.1 mg l^{-1} as stemflow. 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.

CONCLUSION

This investigation suggests that nitrate reduction rates are directly correlated to the organic content of surface sediments within bottomland hardwood wetland soils. Denitrification in such soils seems to be carbon limited. In this case, rates for nitrate reduction were lowest in the frequently flooded tupelo-cypress area (site B4) where the leaf litter was scoured and washed downstream by floodwater. However, the increased duration of flooding in these areas, allowing for longer periods of conditions appropriate for nitrate reduction, may actually result in greater loss of nitrate in frequently flooded areas. The bottomland soils studied also showed that, of the total nitrate reduced, only 5–12 % was contributed by nitrification. This suggests that the processing for nitrate nitrogen in these forested swamps is likely set by the rate and supply of soluble carbon from tree litter and perhaps canopy leachate, rather than by the nitrate concentration in inflowing floodwater.

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